BUENOS AIRES BREAST CANCER SYMPOSIUM
BA-BCS 2021

Mayo, 17-21, 2021

Homenaje a la Dra. Christiane Dosne Pasqualini en su 101 aniversario

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PROGRAM

14:00 **Welcome/Bienvenida**
Claudia Lanari, IBYME-CONICET, Buenos Aires, Argentina
Tribute to doctor Christiane Dosne Pasqualini
Raúl Ruggiero, Academia Nacional de Medicina, Buenos Aires, Argentina

Opening Conference
Chairs: Santiago Bella, Sanatorio Allende y Clínica Universitaria Reina Fabiola, Córdoba, Argentina and José Luis Bocco, CIBICI-CONICET, Córdoba, Argentina
14:15-15:15 **Geoffrey Greene**, University of Chicago, Chicago, USA
"Advances in ER Targeted Approaches to Breast Cancer Management"

Session 1: Tumor heterogeneity and breast cancer therapy
Chairs: Isabel Frahm, Sanatorio Mater Dei, CABA, Argentina and Albana Gattelli, IFIBYNE-UBA-CONICET, CABA, Argentina, Marina Simian, INS-CONICET, San Martín, Argentina
15:30-16:00 **Mohamed Bentires-Alj**, University of Basel, Basel, Switzerland
"Cancer targeted therapy and tumor heterogeneity: Act locally, think globally"
16:00-16:30 **Jorge Reis Filho**, Memorial Sloan Kettering Cancer Center, New York City, USA
"Triple-negative breast cancer subtyping: why bother?"
16:30-16:45 **Catalina Lodillinsky**, Instituto de Oncología Ángel H. Roffo, CABA, Argentina
"Metastasis-suppressor NME1 controls the invasive switch of breast cancer by regulating MT1-MMP surface clearance" (Selected from poster presentations)
16:45-17:00 **Discussion**

Session 2: From hormone receptors to the immune system: the evolution of therapeutic targets in breast cancer
Chairs: Caroline Lamb, IBYME-CONICET, CABA, Argentina; Fernando Petracci, Instituto Alexander Fleming, CABA, Argentina and Cecilia Jazmín Proietti, IBYME-CONICET, CABA, Argentina
17:15-17:45 **Carol Lange**, University of Minnesota, Minneapolis, USA
"Tracking steroid receptor-driven changes in breast cancer cell fate"
17:45-18:15 **Jennifer Richer**, University of Colorado, Aurora, USA
"Breast cancer hijacks a trophoblast-like program of immune suppression"
18:15-18:45 **Mariana Salatino**, IBYME-CONICET, Buenos Aires, Argentina
"Mifepristone primes antitumor immunity in selected luminal mammary carcinomas opening the door to immune therapies"
18:45-19:00 **Andrés Marcos Castellaro**, CIQUIBIC-UNC, Córdoba, Argentina
"Tumor-associated macrophages induce endocrine therapy resistance in ER+ breast cancer cells" (Selected from poster presentations)
19:00-19:15 **Discussion**

Tuesday, May 18th

10:30-13:00 **Poster Session 1**

Session 3: Cancer stem cells and de-differentiated phenotype
Chairs: Gonzalo Gómez Abuin, Hospital Alemán, CABA, Argentina; Mauricio Menacho Márquez, IDICER, CCT-CONICET, Rosario, Argentina and Gastón Soria, CIBICI-CONICET, Córdoba, Argentina
14:00-14:30 **Jochen Maurer**, University Hospital RWTH, Aachen, Germany
"Cancer stem cells as disease models in research-opportunities and challenges"
14:30-15:00 **Paolo Ceppi**, University of Southern Denmark, Odense, Denmark
"The activity of thymidylate synthase shapes the de-differentiated phenotype of aggressive breast cancers"
15:00-15:30 **Robert Clarke**, University of Manchester, Manchester, UK
"Cytokine regulation of stem cell activity, endocrine resistance and metastasis"
15:30-15:45 **Martín Emilio García Solá**, IFIBYNE-UBA-CONICET, CABA, Argentina
"An integrative single-cell transcriptomic atlas of the post-natal mouse mammary gland allows discovery of new developmental trajectories in the luminal compartment" (Selected from poster presentations)
15:45-16:00 **Discussion**

Session 4: Mouse models for studying breast cancer initiation and progression
Chairs: María Marta Facchinetti, INIBIBB-UNS, Bahía Blanca, Argentina and Edith Kordon, IFIBYNE-UBA-CONICET, Buenos Aires, Argentina
16:15-16:45 **D. Joseph Jerry**, University of Massachusetts, Amherst, USA
"Consequences of estrogen exposure among strains of mice: a model for gene and environment interactions"
16:45-17:15 **Fariba Behbod**, University of Kansas, Kansas City, USA
"DCIS progression in the MIND model"
17:15-17:45 **William Muller**, Rosalind and Morris Goodman Cancer Center, Montreal, Canada
"Oncogene-mediated signal transduction in transgenic mouse models of human breast cancer"

17:45-18:00 **Diego Yair Grinman**, Yale School of Medicine, New Haven, USA
"PTHrP overexpression in mammary tumors increases tumorigenesis and causes anorexia" (Selected from poster presentations)
18:00-18:15 **Discussion**

Session 5: Round Table 1 - Genomics Platforms
Chair: Aníbal Nuñez de Pierro, Hospital Fernández, CABA, Argentina; **Co-chairs**: Gustavo Helguera, IBYME-CONICET, CABA, Argentina, Ignacio Mc Lean, Hospital Universitario Austral, Pilar, Argentina
18:30-19:30 **Ernesto Korbenfeld**, Hospital Británico, CABA, Argentina
Fernando Petracci, Instituto Alexander Fleming, CABA, Argentina

Wednesday, May 19th

10:30-13:00 **Poster Session 2**

Session 6: Genetics and Epigenetics of Breast Cancer
Chairs: Martín Abba, UNLP, La Plata, Argentina; Laura Kass, UNL, Santa Fe, Argentina and María Roque Moreno, IHEM-CONICET, Mendoza, Argentina
14:00-14:30 **Adrian Lee**, University of Pittsburgh, Pittsburgh, USA
"Genomics of breast cancer progression"
14:30-15:00 **Sophie Lelievre**, Purdue University College of Veterinary Medicine, West Lafayette, USA
"Environmental epigenetics to fight breast cancer risk and development"
15:00-15:30 **Guenter Vollmer**, Technische Universität Dresden, Dresden, Germany
"Polypharmacology of botanical extracts: Is there a link to breast cancer prevention?"
15:30-15:45 **Santiago Madera**, IBYME-CONICET, CABA, Argentina
"Targeting ErbB-2 nuclear function induces the interferon signalling pathway in breast cancer" (Selected from poster presentations)
15:45-16:00 **Discussion**

Session 7: Understanding the metastatic cascade to learn how to inhibit tumor progression
Chairs: Daniel Alonso, Universidad Nacional de Quilmes, Bernal, Argentina; Enrique Díaz Cantón, CEMIC, CABA, Argentina and Mario Rossi, Universidad Austral, Pilar, Argentina
16:15-16:45 **Valerie Weaver**, University of California, San Francisco, USA
"Tissue force promotes metabolic reprogramming to drive breast tumor aggression and metastasis"
16:45-17:15 **John Condeelis**, Albert Einstein Cancer Center, Bronx, USA
"The mechanism of metastasis during breast cancer progression and how to inhibit it"
17:15-17:45 **Julio Aguirre Ghiso**, Icahn School of Medicine at Mount Sinai, New York, USA.
"The impact of disseminated cancer cell dormancy on the paradigm of metastasis"
17:45-18:00 **Juan Garona**, Universidad Nacional de Quilmes, Bernal, Argentina
"Drug repurposing of hemostatic compound desmopressin (dDAVP) in triple-negative breast cancer (TNBC): Preclinical antitumor activity on 2D/3D cell growth, chemotaxis, tumor progression and metastatic spread" (Selected from poster presentations)
18:00-18:15 **Virginia Judith Wolos**, Instituto de Oncología Ángel H. Roffo, CABA, Argentina
"Hypoxic microenvironment is associated with acquired resistance to HER2+ breast cancer immunotherapies" (Selected from poster presentations)
18:15-18:45 **Discussion**

Session 8: Round Table 2 - Biorepositories and sample management
Chair: Eduardo Sandes, Instituto de Oncología Ángel H. Roffo, CABA, Argentina; **Co-chair**: Fabiana Lubieniecki, Hospital Juan P. Garrahan, CABA, Argentina
19:00-20:30 **Andrea Bosaleh**, Hospital Juan P. Garrahan, CABA, Argentina
Liliana Virginia Siede, UBA-UMSA, CABA, Argentina
Alfredo Molinolo, Moores Cancer Center, UCSD, San Diego, USA
Gonzalo Ardao, Hospital Central de las Fuerzas Armadas (HCFFAA), Montevideo, Uruguay
Ana Palmero, Ministerio de Salud de la Nación, CABA, Argentina

Thursday, May 20th

10:30-13:00 **Poster Session 3**

Session 9: Estrogen receptors: their involvement in endocrine resistance and dormancy
Chairs: Luisa A. Helguero, Institute of Biomedicine (iBiMED), University of Aveiro, Portugal; Isabel Lüthy, IBYME-CONICET, CABA, Argentina
14:00-14:30 **Steffi Oesterreich**, University of Pittsburgh, Pittsburgh, USA
"ER mutations in breast cancer"
14:30-15:00 **Todd Miller**, University of Dartmouth, Lebanon, USA
"Targeting dormancy in ER+ breast cancer"
15:00-15:15 **Discussion**

Session 10: Novel targets in the era of precision medicine
Chairs: Vanesa Gottifredi, Instituto Leloir, CABA, Argentina; Adrián Nervo, Instituto Alexander Fleming, Buenos Aires, Argentina and Virginia Novaro, IBYME-CONICET, CABA, Argentina
15:30-16:00 **Violeta Serra**, Vall d'Hebron Institut d'Oncologia (VHIO), Barcelona, Spain
"CDKs and PARP inhibition in breast cancer"
16:00-16:15 **Santiago Bella**, Sanatorio Allende y Clínica Universitaria Reina Fabiola, Córdoba, Argentina
"Use of CDK inhibitors in South America"
16:15-16:45 **Dejan Juric**, Massachusetts General Hospital, Boston, USA
"News on PI3K inhibitors in clinical practice"
16:45-17:00 **Andrés Elia**, IBYME-CONICET, CABA, Argentina
"Antiproliferative effect of mifepristone in breast cancer patients with higher levels of progesterone receptor A than B: results from the MIPRA trial" (Selected from poster presentations)
17:00-17:15 **Fabiana A. Rossi**, IIMT-CONICET-Univ. Austral, Pilar, Argentina
"USP19 modulates cancer cell migration and invasion and acts as a novel prognostic marker in patients with early breast cancer" (Selected from poster presentations)

17:15-17:30 **Discussion**

Session 11: Round Table 3 - Interaction among government, non-government agencies, and industry for funding and promoting breast cancer translational research
Chair: Omar Coso, IFIBYNE-UBA-CONICET, CABA, Argentina; **Co-chairs**: Edith Kordon, IFIBYNE-UBA-CONICET, CABA, Argentina and Marina Simian, INS-CONICET, San Martín, Argentina
17:45-19:00 **Judith Naidorf**, Facultad de Filosofía y Letras, UBA-CONICET, CABA, Argentina
Rosana Felice, GlaxoSmithKline (GSK), CABA, Argentina
Andrea Llera, Instituto Leloir-CONICET, CABA, Argentina
Daniel Gómez, Universidad Nacional de Quilmes-CONICET, Bernal, Argentina

Friday, May 21st

10:30-13:00 **Poster Session 4**

Session 12: Local and systemic therapies
Chairs: Elisa Bal de Kier Joffe, Instituto Ángel H. Roffo, CABA, Argentina; Pablo Mandó, CEMIC, CABA, Argentina; Roxana Schillaci, IBYME-CONICET, CABA, Argentina
14:00-14:30 **Catherine Park**, University of California, San Francisco, USA
"Radiation oncology-Oligometastasis: The impact of local therapy on systemic disease"
14:30-14:45 **Victoria Costanzo**, Instituto A. Fleming, CABA, Argentina
"Treatment of HER2+ tumors"
14:45-15:00 **Florencia Perazzo**, CEMIC, CABA, Argentina
"Immunotherapy for triple negative breast cancer"
15:00-15:15 **Matthew Winder**, CRUK Beatson Institute, Glasgow, United Kingdom.
"MCL-1 is a clinically targetable vulnerability in breast cancer" (Selected from poster presentations)
15:15-15:30 **Discussion**

Session 13: New developments in diagnosis and epidemiology of breast cancer
Chairs: Roberto Meiss, Academia Nacional de Medicina, CABA, Argentina and Ángela Solano, Facultad de Medicina-UBA, CABA, Argentina
15:45-16:15 **Pedram Razavi**, Memorial Sloan Kettering Cancer Center, New York City, USA.
"cfDNA analysis for breast cancer patients: fact checking"
16:15-16:45 **Osvaldo Podhajcer**. Instituto Leloir, CABA, Argentina
"The Latin American Cancer Research Network: first precision medicine study in human breast cancer in Latin America"
16:45-17:15 **Stephen N Birrell**, The Breast and Endocrine Center, Adelaide, Australia
"Mammographic breast density - the biological and clinical consequences of an opaque breast?"
17:15-17:30 **Gabriela Pataccini**, IBYME-CONICET, CABA, Argentina
"A breast cancer patient-derived xenograft biobank for precision medicine studies in Argentina" (Selected from poster presentations)
17:30-17:45 **Discussion**

Closing Conference
Chairs: J. Silvio Gutkind, UCSD, Moores Cancer Center, San Diego, USA and Pablo Mandó, CEMIC, CABA, Argentina

18:00-19:00 **Charles Perou**, UNC Lineberger Comprehensive Cancer Center, Chapel Hill, USA
"Quantitative Medicine for Breast Cancer Patients"

Closing words
19:00-19:15 **Edith Kordon**, IFIBYNE-UBA-CONICET, CABA, Argentina

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WELCOME

It is an honor and a privilege for me to welcome you on behalf of Edith Kordon, Pablo Mandó, Virginia Novaro, Mario Rossi and Marina Simian to our first Buenos Aires Breast Cancer Symposium 2021. As you all know this Symposium was supposed to be held one year ago at the *Usina del Arte* with the presence of all of you. Optimistically, we thought that in October 2020 we would be recovering our normal life style. However, we had to postpone it once again. Originally we thought that this meeting had to be in an on-site format to achieve the main goals but we had acquired commitments and we finally decided to move to this virtual format.

The main goal of this nonprofit meeting was to expose young Latin American oncologists and investigators to the state of the art in breast cancer research and treatment. In the last years, the possibility of travelling abroad to participate in meetings has been almost prohibitive for scientists and fellows of the region, even before the pandemic. So we thought, if our scientists cannot travel abroad, we have to bring the scientists to our research community, and that was the leitmotif of our project.

First, we would like to thank the speakers from abroad, who had accepted to cover their own travel expenses to Argentina to participate in the symposium. We are really grateful that almost all our original invited speakers accompanied us throughout our changing plans for almost two years. We really hope that we will all be able to meet for real in the second edition of the Buenos Aires Breast Cancer Symposium in 2023.

We are confident that this meeting will greatly strengthen the network among oncologists and basic scientists from different countries, providing opportunities to establish new collaborations among research laboratories and medical centers. We are certain that this meeting, the first of its kind in the region, will significantly contribute to the international effort of fighting breast cancer.

In the program, we have tried to mix basic, translational and clinical lectures in order to integrate scientists and clinicians discussing the most relevant uncertainties about breast cancer. The final balance is favoring basic and translational science, mainly due to the difficulty in recruiting clinical speakers. So, we would like to acknowledge the clinicians who did accept to participate from the very beginning.

We will have two plenary conferences, the opening by Dr. Geoffrey Green and the closing by Dr. Charles Perou, two scientists with an extraordinary professional trajectory with great impact in the breast cancer field, Geoffrey Green developing the first estrogen receptor (ER) antibodies and describing the nuclear ER localization and Charles Perou providing the molecular classification of breast cancer.

The sessions have been organized in 13 thematic mini-symposia with two or three invited speakers and short talks by doctoral or postdoctoral students. These presentations, all unpublished when submitted, have been selected from the abstracts by our Scientific Committee integrated by Drs. Omar Coso, Albana Gattelli, Silvio Gutkind, Alfredo Molinolo, Maria Roque, and Gastón Soria, who we also thank particularly.

All seventy accepted posters will be presented in brief talks during the morning sessions, starting tomorrow. To deepen discussions and increase interactions among participants, all meeting registrants will be able to visit the posters at individual Google meetings hosted by each presenter, at the end of each poster session to discuss the poster with those interested in continue the discussion privately. We cordially invite and encourage all our invited speakers and their fellows to participate in the poster sessions. That would be really important to, at least partially, recreate the atmosphere of a genuine scientific meeting and will help us to achieve our goal of launching an international breast cancer network, not only among established researchers, but also involving the younger generation, which are the future of the field.

In addition, we have organized three thematic round tables to debate genomic platforms, bio-repositories and breast cancer research funding available for breast cancer research in Argentina. These sessions will be held in Spanish because we do not want the language to interfere with the discussion regarding locally available resources in our country or other local regulations of sample managing.

At this point, we would like to acknowledge the pharmaceutical companies that trusted in our meeting from the very beginning: Pfizer, Amgen, Roche, Novartis, and Gador, which also had the flexibility to support us in this virtual format. They showed great enthusiasm of our desire of contributing to bridge the gap between basic and clinical research understanding that this endeavor will result in the benefit of all. We will like to add that although we have sessions sponsored by pharmaceutical companies, they did not have any participation in the content of the talks. In this regard we thank both, speakers for understanding this need, and companies for not interfering with the scientific contents.

Originally we were going to have an open session to the general public with specialists in the field to listen to the queries raised by patients or relatives in order to make improvements that would also reach directly to the society. This had to be cancelled since it was not possible in the virtual format, but we acknowledge MACMA Foundation for trying to make this possible.

We also want to thank the governmental agencies CONICET and the Ministry of Science for their financial support, and the Scientific Societies that contributed with the broadcasting of the meeting: Sociedad Argentina de Investigación Clínica, Sociedad Argentina de Investigaciones Bioquímicas, Sociedad Argentina de Mastología, Sociedad Argentina de Patología, Asociación Argentina de Oncología Clínica and the Instituto Nacional del Cáncer.

Since many registrants had already paid to attend to the original version of this meeting, we found ourselves

in the need to compensate them when we moved to this virtual format. Since we were not able to make cash refunds, several companies have donated laboratory disposable supplies that will be used to fairly compensate for the received payments. For their help on this issue, we want to acknowledge Lobov, Microlat, GBO and Ap-Biotoech, Argentina.

We particularly wish to thank Silvina Ceriani and her team as well as Fundación IBYME, which were in charge of the organization and financial issues related with the meeting, for their excellent job.

We are confident that this meeting will be a success, and we hope for the next one to have more participation of other Latin American specialists. In fact, we welcome any suggestions directed to improve the interaction between Latin American countries, which we know that are underrepresented in this meeting.

All the abstracts from oral and poster sessions will be published in a special Supplement of *Medicina (Buenos Aires)*, an open access journal that is indexed in PubMed and Scopus.

We have also decided to honor one of our pioneer scientists in the cancer field that last year had her 100 birthday, and now she is 101 years old. She is Christiane Dosne Pasqualini. She was born in France, brought up in Canada and was a student of Hans Selye. She came to work with Dr. Bernardo Houssay, a Nobel Prize winner, for a short term, but she married in Argentina and decided to remain here the rest of her life, without giving up her career. She was my mentor and two other members of the organizing committee, Edith and Marina, also started their careers in her Laboratory. We have asked two of her disciples, Dr. Raul Ruggiero, who has worked for more than 40 years with her, and Dr. Oscar Bustuoabad, who collaborated with her during the last years, to write an article that will be published in the same supplement. So Dr. Christiane Pasqualini, this is in your honor.

To end, we hope that this meeting might be the beginning of a new step for breast cancer research in Argentina, where the scientific and the medical communities can advance together towards an improvement in the diagnosis, treatment and management of this disease, tasks that in developing countries like ours, are more affected for various reasons. We trust that the community, the authorities and companies can find a way to work together with this common goal.

So please enjoy the meeting, we hope internet and electricity will be with us all the time, and we look forward to have an excellent week. So thank you very much to all of you, speakers, chairs and attendees.

Claudia Lanari on behalf of the Organizing Committee

Tribute to doctor Christiane Dosne Pasqualini

There are people who have the rare fortune to know someone that, over the years, will become an unforgettable figure of their lives. We had that fortune because we met Dr. Christiane Dosne Pasqualini.

Christiane Dosne Pasqualini was born in France in 1920. She was the eldest of four brothers and sisters. At the age of 6, her family moved to Canada. In that country she graduated, at the age of 22, as PhD in Experimental Medicine under the direction of Dr. Hans Selye, the great Hungarian-Canadian endocrinologist (nominated for the Nobel Prize in Physiology or Medicine in 1949) who coined the universally accepted term “stress” to describe the body’s method of reacting to different noxious agents. That same year (1942), Christiane got a scholarship to work in Buenos Aires, Argentina at the *Instituto de Fisiología de la Universidad Nacional de Buenos Aires* under the direction of Dr. Bernardo A. Houssay (Nobel Prize in Physiology or Medicine in 1947). In that institute, Christiane worked alongside with Dr. Luis F. Leloir (Nobel Prize in Chemistry in 1970), Dr. Alfredo Lanari (father of Claudia, one of the organizers of the BA-BCS 2021) and Dr. Rodolfo Pasqualini, amongst many other relevant clinicians and scientists.

In 1944 Christiane was awarded a scholarship to work at Yale University. At the end of that year, she married Rodolfo Pasqualini, one of her former colleagues at the *Instituto de Fisiología de Buenos Aires*, with whom she had five children. After her marriage, Christiane settled definitively in Argentina, obtained the Argentinian citizenship and began to work at the *Instituto de Endocrinología* directed by her husband.

In 1957 Christiane entered the National Academy of Medicine and began to be interested in the causes of cancer. In 1962, she was accepted as a member of the Research Career of the *Consejo Nacional de Investigaciones Científicas y Técnicas* (CONICET), created a few years earlier by her former boss, Bernardo Houssay. Four years later (1966), she was appointed as head of the *Sección Leucemia Experimental* at the *Academia Nacional de Medicina* de Buenos Aires. From that place and for the next 50 years she carried out a tireless and fruitful work in the study of cancer not only directing a countless number of fellows and researchers but also as a member of the editorial committee of the scientific and medical journal *Medicina (Buenos Aires),* and as President of different scientific societies. In 1991, Christiane was the first woman to be incorporated as full member at the *Academia Nacional de Medicina de Buenos Aires.* In 1995 she received in Los Ángeles, USA, the international UNIFEM/Noel award aimed at highlighting relevant female personalities throughout the world. She worked uninterruptedly until she was 95 years of age. Since then she has taken a deserved long vacation, although, at her 101 years, she still welcomes her numerous descendants and many of her former collaborators to her home.

She has written multiple scientific and autobiographic books as well as more than 100 scientific papers, many of them published in the most prestigious international scientific journals. These papers cover very diverse topics such as the role of adrenals in the general resistance to stressors, the effect of ascorbic acid in relieving hemorrhagic shock in guinea pigs, the leukemogenic and sterilizing effect of radioactive phosphorus in mice and humans, the model of glass cylinder in mice that allows the growth of allogeneic tumors, the study of oncogenic virus in mice and humans, the involvement of superantigens in putative strategies aimed to prevent the growth of murine leukemias, the phenomenon of concomitant tumor resistance by which tumor-bearing individuals can inhibit the growth of secondary tumors experimentally or spontaneously (metastases) implanted far from the primary tumor, the influence of hormones such as progesterone and estrogens in mammary carcinogenesis in both mice and humans, among many other research subjects.

Up to this point we have made a short and objective biographical sketch of Dr. Christiane D. Pasqualini. However, now, we would like to offer a more personal view of her and the Department of Experimental Leukemia that she directed when we got there almost at the same time near the end of the 70’s and the beginning of the 80’s.

Seated from left to right: Raúl A. Ruggiero, Christiane Pasqualini and Graciela Dran. Standing up from left to right: Damián Machuca, Juan Bruzzo Iraola, María Noel Badano, Andrea Maglioco y Paula Chiarella. All comprised the Laboratorio de Oncología Experimental in 2015. This is the last photograph of Christiane in the laboratory, at the age of 95.

She was a tall and imposing woman and her Department was the most beautiful and friendly place to work that we ever can remember. We were about 25-30 persons amongst researchers, fellows and technicians. Most of us were very young but we were able to publish our papers in the best scientific journals of that time. In addition, and most importantly, there was a climate of camaraderie and friendship among all of us that easily allowed the exchange of ideas, experimental technics, reagents and mice. And Christiane was behind everything, supporting everyone, although probably neither one of us was fully aware of it. She encouraged us to work tirelessly and to write our results and to present them in national or international meetings and finally to publish them in the appropriate journals. And, especially, she stimulated us to work and to think as freely as possible, not abiding by the dogmas or the established precepts, always trying to go beyond the obvious into remote and unknown lands. Her energy was never tinged with nostalgia. She never turned around and looked back wistfully even though she had met and worked with the best scientists of her time. No, she always looked forward to the future, enthusiastically, trying to make us feel like her, what she called the joy of living [joie the vivre] for that time full of work and hope.

Perhaps nothing better exemplifies her preaching than two sentences of Albert Einstein that were posted in her laboratory. The first one said: “The most beautiful experience we can have is the mysterious. It is the fundamental emotion that stands at the cradle of true art and true science. Whoever does not know it and can no longer wonder, no longer marvel, is as good as dead, and his eyes are dimmed”. The other sentence said: “If at first an idea is not absurd, then there is no hope for it”.

Dr. Christiane D. Pasqualini always urged us to keep our eyes and our minds open. What we did afterwards was solely our responsibility.

For us, her direct disciples, there is only a feeling of gratitude for all that we received from her over the last 60 years. We can say today, without any doubt, –mimicking and adapting a dedication of Carl Sagan to his wife–, that in the vastness of space and the immensity of time, it is our joy to have shared a planet and an epoch (golden and distant) with Christiane.

Dr. Raúl A. Ruggiero
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Closing words (excerpt* from farewell talk)

Good evening everybody, I’ve got the sad task to give the farewell words on behalf of the organizers of this meeting. It is a bitter-sweet moment, as we are also very happy because we feel that our mission has been accomplished. In fact, the success of the BA-BC S2021 has exceeded our own expectations.

As you can imagine the organization of the first Buenos Aires Breast Cancer Symposium has been a roller-coaster. Many things changed and many remained the same since Claudia shared with me her idea of organizing

*Authors’ note: In this shortened version of our farewell words, we have excluded the commentary about the content of the conferences, mini-symposia and round tables, as abstracts of all these presentations and activities are included in this Supplement

an international Breast Cancer Meeting in Buenos Aires. For this, we first met Silvina Ceriani, almost 3 years ago, and soon after Pablo Mandó, Virginia Novaro, Mario Rossi and Marina Simian joined us. We began by discussing the program, location, invited speakers and our scientific committee. As we knew we would not be able to raise enough money to cover travel expenses for all speakers, we sent our invitations making sure our proposed guests understood our budget limitations. Fortunately, they were all happy to travel to Buenos Aires in May 2020, at their own cost. So, by mid-2019 we had chosen the *Usina del Arte* as the venue for our meeting, a beautiful, huge historical building in the traditional La Boca district in Buenos Aires. In December 2019 registration was opened. We had more than 80 registrants by the end of the year (four and a half months before the meeting), so everything looked bright and promising.

But you all know what happened between February and March 2020. In April we were optimistic enough to postpone our meeting to October. Almost all speakers agreed on the new dates, but, of course, we had to cancel once again. By November, we decided that we had to move to a virtual format. Once again, we wrote to all our original speakers and most of them accepted this new format.

So, when I said that many things had changed but many remained the same since we started, I was actually referring that throughout these exceptional times the whole organizing committee, Silvina Ceriani, Fundación IBYME, our scientific committee and the vast majority of our invited speakers and chairs remained associated to the project. We are very grateful because that support made this meeting possible, even in the middle of the worst world-wide pandemic of all times.

But, here we are! Very happy, and very tired, finishing an amazing meeting! Already in 2019 we announced that this was going to be the first breast cancer meeting of its kind in the region, but we did not expect it to be so unique.

During these 5 days we enjoyed 2 lectures, 27 talks from invited speakers in the mini symposia, 3 round tables and 81 posters, 11 of which were selected for oral presentations. The other accepted abstracts were presented in 4 sessions, each of which had between 85 to 110 attendees from different labs from all around our country, and from abroad (*i.e.* Chile, Portugal, USA and Scotland, UK). We thank all poster presenters and the audience for the lively debates. We are particularly grateful to the 12 poster coordinators, for their excellent job as slide operators and translators in addition to the regular chair´s duties as presenters and discussion moderators. We think that the poster sessions were one of the highlights of the BA-BCS 2021 and it will be a challenge for us to gather such participative and broad audiences in future “face to face” meetings.

A few last words referred to this virtual format. We are very grateful to all chairs, coordinators and speakers for their terrific work, dealing with the unexpected problems related to network connectivity. And we also want to thank Paul Caballero, our web page and platform designer, for his excellent job.

Finally, we also want to express our gratitude to all our 400 registrants for their participation and for being important actors in the generation of interactive discussions in all sessions and for all the positive and supportive comments received throughout the meeting.

Well…I guess it is time to say good bye…We hope next time we will be able to meet face to face and share barbeques, vegetarian or vegan food, empanadas, pizza, pasta, local wines and beers…etc. We have a lot to offer in that aspect! And in the meantime, stay tuned, since from here we would like to start a new international breast cancer research network that will give us the possibility to keep in touch in spite of the distance. You will all receive an email from us soon, with your certificate of assistance and also with information related to this new network.

So, so-long, farewell and keep in touch

Edith Kordon on behalf of the Organizing Committee

OPENING CONFERENCE

Advances in ER Targeted Approaches to Breast Cancer Management

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For more than 50 years, estrogen receptor alpha (ER) has been widely used as a predictive and prognostic biomarker in breast cancer. ER is also a well-established therapeutic target and numerous drugs have been developed that target ER, both indirectly (AIs, aromatase inhibitors) and directly as tissue-selective ER inhibitors, falling into two general categories: SERMs (selective ER modulators) or SERDs (selective ER degraders). A major unresolved clinical issue is the development of endocrine therapy (ET) resistance, which invariably occurs during prolonged adjuvant ET. A significant contributing mechanism by which tumors progress is through the acquisition of activating mutations in the ESR1 gene, most notably in the ERα ligand-binding domain. We, and others, have observed somatic ESR1 mutations in up to 40% of metastatic tumors obtained from women who have acquired resistance to various endocrine therapies, especially to AIs. The two most common mutations are Y537S and D538G, both of which stabilize and/or facilitate the formation of an active AF-2 conformation in the ER LBD. A combination of structural, biophysical, cell and animal studies have helped define the underlying molecular mechanisms that account for AI/SERM/SERD resistance related to ESR1 mutations, which has contributed to the development of next generation SERMs and SERDs with potential improved clinical utility. Several of these drugs are currently being tested in clinical trials.

Sessions

Session 1 - Tumor Heterogeneity and Breast Cancer Therapy

Cancer targeted therapy and tumor heterogeneity: Act locally, think globally

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Each year over 2.1 million new cases of breast cancer occur among women worldwide and 600,000 women die from this disease. In most cases, metastasis is the cause of death. Indeed, while 98% of patients survive 5 years or more after being diagnosed with a localized (confined to the primary site) breast cancer, this number drops to 15-25% if the cancer has metastasized to distant organs. Curing metastatic breast cancer clearly represents an unmet medical need.

Although progress has been made in broadly understanding breast tumor biology and progression to metastases, most of the relevant molecules and pathways remain undefined. The thread connecting the research in my lab is tumor heterogeneity. We assess mechanisms that influence normal and neoplastic breast stem cells, metastasis, and resistance to targeted therapies at the molecular, cellular, and whole organism levels considering both cell autonomous and non-cell autonomous mechanisms. We have also developed a personalized breast cancer treatment program.

Triple-negative breast cancer subtyping: why bother?

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No abstract available

Metastasis-suppressor NME1 controls the invasive switch of breast cancer by regulating MT1-MMP surface clearance

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Membrane Type 1 Matrix Metalloprotease (MT1-MMP) contributes to the invasive progression of breast cancers by degrading extracellular matrix tissues. Nucleoside diphosphate kinase, NME1/NM23-H1, has been identified as a metastasis suppressor; however, its contribution to local invasion in breast cancer is not known. Here, we report that NME1 is up-regulated in ductal carcinoma in situ (DCIS) as compared to normal breast epithelial tissues. NME1 levels drop in microinvasive and invasive components of breast tumor cells relative to synchronous DCIS foci. We find a strong anti-correlation between NME1 and plasma membrane MT1-MMP levels in the invasive components of breast tumors, particularly in aggressive histological grade III and triple-negative breast cancers. Knockout of NME1 accelerates the invasive transition of breast tumors in the intraductal xenograft model. At the mechanistic level, we find that MT1-MMP, NME1 and dynamin-2, a GTPase known to require GTP production by NME1 for its membrane fission activity in the endocytic pathway, interact in clathrin-coated vesicles at the plasma membrane. Loss of NME1 function increases MT1-MMP surface levels by inhibiting endocytic clearance. As a consequence, the ECM degradation and invasive potentials of breast cancer cells are enhanced. This study identifies the down-modulation of NME1 as a potent driver of the in situ-to invasive transition during breast cancer progression.

Session 2 - From Hormone Receptors to the Immune System: The Evolution of Therapeutic Targets in Breast Cancer

Tracking Steroid Hormone Receptor Driven Changes in Breast Cancer Cell Fate

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Steroid hormone receptors (SRs) are classically defined as ligand-activated transcription factors that function as

master regulators of gene programs important for a wide range of physiological processes governing cell or tissue homeostasis. As such, dysregulated SRs are targeted drivers of cancer cell proliferation. A second function of SRs includes their ability to rapidly activate cytoplasmic signaling pathways. In addition to making direct contact with diverse signaling molecules, SRs are fully integrated with signaling pathways by virtue of their N-terminal phosphorylation sites that act as regulatory hot-spots capable of sensing the signaling milieu. In particular, ER, PR, and closely related glucocorticoid receptors (GR) share the property of accepting (i.e. sensing) ligand-independent phosphorylation events by proline-directed kinases in the MAPK and CDK families. These signaling inputs act as a 'second ligand' that dramatically impacts cell fate. In the face of drugs that target SR ligand-binding domains to block proliferation, ligand-independent post-translational modifications alter SR-binding partners to guide changes in cell fate that confer increased survival, migration, stemness properties, and therapy resistance of SR+ cancer cells. Targeting phospho-SRs in addition to the key mediators of phosphorylation-dependent changes in breast cancer cell fate will be an impactful addition to modernized combination therapies.

Breast Cancer Hijacks a Trophoblast-Like Program of Immune Suppression

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TNBC cells rapidly change their metabolism during anchorage independent survival. Proteins that increase during anchorage independent survival include LAT1, a transporter of large amino acids such as tryptophan. In TNBC tryptophan catabolism is largely mediated by the enzyme tryptophan-2,3-dioxygenase (TDO2). During embryonic development epithelial cells undergo epithelial to mesenchymal transition (EMT) to accomplish normal developmental events. Fetal trophoblasts undergo an EMT that facilitates invasion into the uterus and suppression of the maternal immune system to ensure fetal tolerance during pregnancy. Carcinomas undergo EMT to facilitate anchorage independence, invasion, and metastasis, but less is known regarding how oncogenic EMT facilitates tumor cell immune evasion. Restoration of the microRNA miR-200c to TNBC powerfully reverses EMT and thereby reveals exact mechanisms by which TNBC suppress the anti-tumor immune response. We find that miR-200c directly targets and represses TDO2, HMOX-1, IKBKB, PLCG1, and PD-L1 and other targets involved in immune suppression. Our findings provide insights into targetable transcriptional and post-transcriptional regulation of immuno-suppressive factors produced by TNBC. The data support our hypothesis that metabolic alterations in TNBC result in production of immune-suppressive metabolites, and that targeting these pathways may help prevent or contain metastases by boosting immune cell function.

Mifepristone primes antitumor immunity in selected luminal mammary carcinomas opening the door to immune therapies

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The role of active antitumor immunity in hormone receptor-positive (HR+) breast cancer has been historically underlooked. We are interested in evaluating the contribution of the immune system to antiprogestin-induced tumor growth inhibition using a hormone-dependent breast cancer model. We have generated a mouse model by transplanting BALB/c-GFP+ bone marrow (BM) cells into immunodeficient NSG mice recipients to generate an immunocompetent NSG/BM-GFP+ (NSG-R) reconstituted model. Treatment with the antiprogestin Mifepristone (MFP) inhibited the growth of 59-2-HI tumors in immunocompetent or immunocompromised mice. However, in immunocompetent mice MFP treatment reshaped the tumor microenvironment, enhancing the production of proinflammatory cytokines and chemokines. Tumors showed increased infiltration of F4/80+ macrophages, NK, and CD8 T cells, displaying a central memory phenotype. Mechanistically, MFP induced an immunogenic cell death gene program, activating as a consequence immature dendritic cells, and induced a memory T cells response, that attenuates tumor onset and growth after re-challenge. Finally, MFP treatment increased the sensitivity of HR+ 59-2-HI tumor to PD-L1 blockade, suggesting that antiprogestins may improve immunotherapy response rates. Our work contributes to better understand the mechanisms underlying the antitumor effect of hormonal treatment in selected luminal mammary carcinomas opening the door to immune therapies.

Tumor-Associated Macrophages Induce Endocrine Therapy Resistance in ER+ Breast Cancer Cells

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Antiestrogenic adjuvant treatments are first-line therapies in patients with breast cancer positive for estrogen receptor (ER+). But most patients eventually acquire endocrine resistance and many others are initially refractory to anti-estrogen treatments. The tumor microenvironment plays essential roles in cancer development and progression; however, the molecular mechanisms underlying such effects remain poorly understood. Breast cancer cells lines co-cultured with TNF-α-conditioned macrophages were used as pro-inflammatory tumor microenvironment models. Proliferation, migration, and colony formation assays were performed to evaluate tamoxifen and ICI182780 resistance and confirmed in a mouse xenograft model. In our simulated pro-inflammatory tumor microenvironment, tumor-associated macrophages promoted proliferation, migration, invasiveness, and breast tumor growth of ER+ cells, rendering these estrogen-dependent breast cancer cells resistant to estrogen withdrawal and tamoxifen or ICI 182780 treatment. Crosstalk between breast cancer cells and conditioned macrophages induced sustained release of pro-inflammatory cytokines from both cell types, activation of NF-κB/STAT3/ERK, and hyperphosphorylation of ERα in the cancer cells, which resulted in constitutively active. Our simulated tumor microenvironment strongly altered endocrine and inflammatory signaling pathways in breast cancer cells, leading to endocrine resistance in these cells.

Session 3 - Cancer Stem Cells and De-differentiated Phenotype

Cancer stem cells as disease models in research - opportunities and challenges

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Triple negative breast cancer is a highly aggressive subtype of breast cancer with 9,000 - 14,000 new cases in Germany each year and is characterized by a significant higher frequency of visceral as well as central nervous system metastases than the other subtypes. Due to this clinical behaviour TNBC shows a poor prognosis profile with significant lower recurrence free and overall survival rates, respectively. Breast cancer stem cells (BCSCs) are hypothesized to play a crucial role for the tumor biological behavior of TNBC with the high metastatic potential as well as the observed metastatic pattern. We established a method to isolate and propagate BCSC from individual triple-negative tumors resected from patients after neoadjuvant chemotherapy and characterized the cells in vitro and in vivo. We utilize BCSCs in analyzing basic tumor biology and try to model the interaction of different cell types in tumor tissue. Since already a few of these cells are capable of recapitulating the patient tumor with matching histology and gene expression in immunocompromised animals, we can use the cells also for screening and testing novel therapeutics. As a proof-of-concept we describe an orally available, selective and potent KDM4 inhibitor (QC6352) with unique preclinical characteristics. QC6352 blocked BCSC proliferation, sphere formation and xenograft tumor formation. QC6352 also abrogated expression of EGFR which drives the growth of therapy-resistant triple-negative breast cancer cells.

The activity of thymidylate synthase shapes the de-differentiated phenotype of aggressive breast cancers

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Cancer cells undergo dramatic shift in metabolism to overcome the bioenergetic and proliferative stress. One of the key changes is the increased expression of nucleotide metabolism genes (NMGs), believed to support the excessive demand of deoxynucleotides for proliferation and nucleotide based co-factors (e.g. NADP, NDAPH etc.) for cellular energetics. However, the concept of extracellularly generated purinergic signaling triggered by purine molecules provided the prime evidence that nucleotides can have non-proliferative functions in cancer. Our lab recently showed the role of pyrimidine metabolism (PyM) in epithelial cell plasticity by linking the activity of PyM genes thymidylate synthase (TS) and thymidine catabolism to the differentiation status of triple negative breast cancer (TNBC). Expressed at a comparatively higher level in de-differentiated breast cancers, TS generates excessive thymidylate (dTMP) that is fluxed in the catabolic pathway regulated by dihydropyrimidine dehydrogenase (DPYD) to maintain a de-differentiated EMT-like state. Importantly, our data show that although TS mediated differentiation is dependent on enzymatic activity, it is completely independent of its role in proliferation. TS ablation in TNBC cell lines mitigated the stem-like phenotype and the loss of EMT in TS-deficient cells was confirmed by RNA sequencing and in vivo work. Overall, these results provide a strong rationale to further explore the effect of PyM on cancer differentiation.

Cytokine regulation of stem cell activity, endocrine resistance and metastasis

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Metastatic ER+ breast cancer is treated with endocrine therapies, and more recently CDK4/6 inhibitors. In order to discover resistance mechanisms contributing to growth in metastatic sites and progression, we studied the tumour cells that survive anti-estrogen therapies such as tamoxifen and fulvestrant. We found that tamoxifen and fulvestrant therapy-resistant cells have cancer stem cell (CSC) attributes such as aldehyde dehydrogenase (ALDH) enzyme activity, mammosphere colony formation ex vivo and tumour initiation in vivo. We established that these CSC activities are regulated by pathways downstream of the interleukin (IL) 1beta/IL1 receptor, and IL6/STAT3 signalling. These cytokine signalling pathways contribute to both metastatic progression and endocrine and CDK4/6 inhibitor therapy resistance. Targeting them in combination with current therapies has the potential to improve clinical outcomes.

An integrative single-cell transcriptomic atlas of the postnatal mouse mammary gland allows discovery of new developmental trajectories in the luminal compartment

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The mammary gland (MG) is a highly dynamic organ which undergoes periods of expansion, differentiation and cell death in each reproductive cycle. In agreement with the dynamic nature of the gland, mammary epithelial cells (MECs) are extraordinarily heterogeneous. Single cell RNA-seq (scRNA-seq) analyses have contributed to understand the cellular and transcriptional heterogeneity of this complex tissue. Here, we integrate scRNA-seq data from three foundational reports that have explored the MG cell populations throughout development at single-cell level. We focused our analysis on MG post-natal development. This new integrated study corresponds to RNA sequences from a total of 53,686 individual cells. The large volume of information provides new insights, as a better resolution of the previously detected Procr+ stem-like cell subpopulation. Moreover, our study proposes new pseudo-temporal trajectories of MEC populations at two resolution levels, either considering all mammary cell subtypes or focusing specifically on the luminal lineages. Interestingly, the luminal-restricted analysis reveals distinct expression patterns for different millk-protein genes, suggesting specific and non-redundant roles for each of these proteins. In summary, our data show that the application of bioinformatic tools to integrate multiple scRNA-seq data-sets helps to describe and interpret the high level of plasticity involved in gene expression regulation throughout MG post-natal development.

Session 4 - Mouse Models for Studying Breast Cancer Initiation and Progression

Consequences of estrogen exposure among strains of mice: a model for gene and environment interactions
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Estrogens together with progestins play critical roles in development of breast tissue, but also contribute to the risk of breast cancer. Twin studies reveal that 30% of breast cancer risk can be attributed to inherited genetic variants with the remaining risk associated with the environment and include differences in reproductive factors and environmental exposures. But the mechanisms by which exposure to endogenous hormones and environmental chemicals may interact with inherited risk remains unclear. Previous studies demonstrated that mammary tumors develop spontaneously in BALB/c mice with heterozygous mutations in the p53 tumor suppressor gene (BALB/c-Trp53+/-) providing a model of Li-Fraumeni Syndrome. However, C57BL/6-Trp53+/- mice rarely develop mammary tumors. We mapped the genetic modifiers and created mice that are congenic for C57BL/6 alleles within a 20Mb interval (SM1-Trp53+/-) on mouse chromosome 7. In contrast to BALB/c-Trp53+/- mice, the C57BL/6 alleles in SM1-Trp53+/- mice were sufficient to reverse repair of DNA double strand breaks by low-fidelity pathways and enhance processivity of replication forks. These strains also differ in their susceptibility to estrogen-induced DNA damage. The results show that the combined effects of estrogen-induced DNA damage and genetic modifiers regulating the fidelity of DNA repair interact to alter the incidence of mammary tumors in mice.

Mouse-INtraDuctal (MIND): An in vivo model that recapitulates the full spectrum of human DCIS pathology
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Due to an increase in screening mammography, there has been a significant increase in the rate of ductal carcinoma *in situ* (DCIS) diagnosis. At the present time, there are no means by which to diagnose DCIS accurately, or predict which patients require aggressive therapy. To address this gap, we present the first *in vivo* model, referred to as Mouse-INtraDuctal (MIND), by which patient-derived DCIS epithelial cells are injected intraductally and allowed to progress naturally in mice. Similar to human DCIS, the cancer cells form *in situ* lesions inside the mouse mammary ducts and mimic all histologic subtypes including micropapillary, papillary, cribriform, solid, and comedo. After a median of 9 months, 15/35 (42%) patient samples injected into 95 xenografts remained non-invasive; 20/35 (57%) patient samples injected into 95 xenografts advanced to invasive lesions. While there was some level of discordance between patients and xenografts with regards to the expression of clinically relevant biomarkers, only progesterone receptors showed a significant correlation to invasive progression. Targeted sequencing of LCM-captured DCIS DNA from patient/xenograft pairs found a number of common, unique and private pathogenic mutations. In summary, MIND models are valuable resources for the discovery of patient-specific molecular signatures of DCIS invasiveness through the comprehensive analysis of patient-derived xenografts with variable propensity for malignancy.

Oncogene-mediated signal transduction in transgenic mouse models of human breast cancer
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Mechanistic Target of Rapamycin Complex 1 (mTORC1) is a master modulator of cellular growth, and its aberrant regulation is recurrently documented within breast cancer. While the small GTPase Rheb1 is the canonical activator of mTORC1, Rheb1-independent mechanisms of mTORC1 activation have also been reported but have not been fully understood. Employing multiple transgenic mouse models of breast cancer, we report that ablation of Rheb1 significantly impedes mammary tumorigenesis. In the absence of Rheb1, a block in tumor initiation can be overcome by multiple independent mutations in Mtor to allow Rheb1-independent reactivation of mTORC1. We further demonstrate that the mTOR kinase is indispensable for tumor initiation as the genetic ablation of mTOR abolishes mammary tumorigenesis. Collectively, our findings demonstrate that mTORC1 activation is indispensable for mammary tumor initiation, and that tumors acquire non-canonical mechanisms of mTORC1 activation.

PTHrP overexpression in mammary tumors increases tumorigenesis and causes anorexia
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Cancer Anorexia Cachexia Syndrome (CACS) is a life-threatening complication of disordered energy metabolism that afflicts 26% of all patients with breast cancer. CACS is caused by the tumor release of soluble factors leading to pronounced loss of weight, appetite, skeletal muscle and fat mass. Importantly, breast cancer patients with weight loss are more resistant to and less tolerant of chemotherapy and surgery. One of the secreted factors implicated in CACS is PTHrP. We have developed an inducible transgenic mouse model that overexpresses PTHrP in PyMT-derived mammary tumors (Tet-PTHrP;PyMT). Administering Dox to Tet-PTHrP;PyMT mice activated human PTHLH cDNA expression in mammary tumors, leading to elevated circulating PTHrP levels and accelerated tumor growth. Tet-PTHrP;PyMT mice on Dox also experienced hypercalcemia along with a profound anorexia, rapid fat wasting and weight loss. Pair feeding experiments demonstrated that anorexia is the main driver of weight loss in our model. Moreover, we found that PTHrP overexpression caused a significant activation of the lateral parabrachial nucleus (LPBN), an area of the brain involved in appetite regulation. Finally, blocking the PTHrP induced bone resorption with Osteoprotegerin corrected the hypercalcemia and prevented the anorexia and the LPBN activation. Overall, our data suggest that mammary tumor expression of PTHrP increases tumor burden and, in parallel, causes anorexia possibly through a calcium dependent mechanism.

Session 5 - Round Table 1 - Genomics Platforms
In Spanish at the end as a special article

Session 6 - Genetics and Epigenetics of Breast Cancer

Genomics of breast cancer progression
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Advances in sequencing and associated bioinformatic analyses are allowing unprecedented insight into how breast cancers become drug resistance and progress to metastasis. We are analyzing breast cancer progression by sequencing patient-matched pairs of tumor biopsies (primary and metastasis), longitudinal circulating tumor DNA (ctDNA) and post-mortem tissue. Through this program we have identified numerous gained attributes in metastatic disease with some of these representing potential therapeutic opportunities. In this talk I will summarize our work to date in this area.

Environmental epigenetics to fight breast cancer risk and development
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The multifactorial origin of cancer is associated with the build-up of epigenetic changes that ultimately push cells into the cancer spiral. The underlying mechanisms involve environmental exposure-mediated alterations in the microenvironment of cells. Exosomes and free molecules, like reactive oxygen species, are likely contributors to the microenvironmental impact on the epigenome, in addition to physical constraints. A major issue in prevention is to identify worthwhile biological risk markers of a particular cancer that may also be early indicators of the success of a preventive intervention. Another issue is to revert the risk by targeting the appropriate epigenetic pathways, with methods that include as little as possible disadvantage for the patient. These are some of the goals of the transdisciplinary International Breast Cancer & Nutrition (IBCN) Project. I will present collaborative approaches to identify epigenetic markers of risk that combine different risk populations and *in vitro* risk-on-chip (ROC) models. The ROC can be tailored to specific microenvironmental conditions of human tissues. Results will be discussed in the context of the accelerated aging of tissues and the modulatory effect of nutrition on the exposome.

Polypharmacology of botanical extracts: Is there a link to breast cancer prevention?
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Breast cancer is the most frequently diagnosed cancer occurring in women in Germany with almost 72,000 cases diagnosed in 2013 and an expected increase to 77,000 cases in 2020. It is unquestionable that estrogens play a pivotal role in the development of breast cancer, as about 70% of all breast cancers cases are estrogen dependent, with four major mechanisms contributing to estrogen-dependent mammary gland carcinogenesis and breast cancer growth. These are the hormonal, the chemical, the inflammatory, and the epigenetic pathway. Consequently, inhibition of any of these pathways may result in breast cancer prevention. So far, we focused on the hormonal pathway, which plays a key role in tumor promotion by estrogenic compounds through an estrogen receptor-α (ERα) dependent mechanism. While conventional therapies focus on the inhibition of synthesis of the ligand E2 or on inhibition of its function on the ERα by receptor antagonists, we hypothesize that the polypharmacological nature of botanical extracts may functionally inhibit ERα by simultaneously activating additional/alternative pathways, which in turn functionally inhibit ERα mediated effects. Those pathways comprise the ERα signaling and the arylhydrocarbon receptor (AhR) signaling pathway. The examples provided here suggest that activation of the ERα- or the AhR-signalling pathways by non-toxic, plant-derived agonists may represent a preventive strategy for hormone dependent mammary gland tumors.

Targeting ErbB-2 nuclear function induces the interferon signalling pathway in breast cancer
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ErbB2, a member of ErbB family of receptor tyrosine kinases, is an oncogenic driver in breast cancer (BC). Despite clinical efficiency of ErbB2-targeted therapies (trastuzumab) resistance to drugs is a major issue. ErbB2 is a membrane-bound receptor, but also migrates to the nucleus (NErbB2) to act as a transcription factor/coactivator. We reported the paramount importance of NErbB2 in TZ-resistant BC; now we used a TZ-resistant model with high basal NErbB2 levels to explore its induced transcriptome. RNAseq was run on JIMT1 cells transfected or not with an ErbB2 nuclear localization domain mutant which is also a dominant-negative inhibitor of endogenous NErbB2 migration. Exclusion of NErbB2 modulated the expression of nearly 300 genes. Functional analysis revealed that NErbB2 blockade enriched the expression of genes involved in type-I interferon signaling pathway. IFNB1, OAS2 and TRIM22 were among the top modulated genes. In independent validation experiments blockade of NErbB2 induced expression of these genes in different models of BC. *In vivo* experiments demonstrated that blockade of NErbB2 significantly inhibits tumor growth and induced mRNA expression of these genes. ChIP assays revealed recruitment of ErbB2 onto IFNB1 coding and promoter regions in normal growth conditions. These results reveal the repression of the type-I interferon pathway as a mechanism of carcinogenesis in ErbB2positive BC and essentially highlight NErbB2 as a therapeutic target in TZ-resistant BC.

Session 7 - Understanding the Metastatic Cascade to Learn how to Inhibit Tumor Progression

Mechanosensitive hormone signaling promotes mammary progenitor expansion, cancer risk and malignant progression
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Tissue stem cell frequency has been implicated in risk to malignancy and tumor aggression. Tissue-spe-

cific factors linking stem cell frequency to cancer risk and progression remain ill defined. Using a genetically engineered mammary mouse model of elevated integrin mechanosignaling, and syngeneic orthotopic manipulations and patient-derived breast tumor xenografts implanted within cross-linked collagen we observed that a stiff extracellular matrix (ECM) and high integrin mechanosignaling increased the number of epithelial cells with a basal-like mesenchymal phenotype and promoted breast tumor metastasis. A stiffened ECM and high integrin mechanosignaling also increased mammary progenitor cell frequency to enhance breast tumor initiation. Upon further investigation we determined that a stiff ECM and high integrin- mechanosignaling potentiated progesterone receptor-dependent RANK signaling that expanded breast epithelial stem/progenitor frequency. Consistently, inhibiting RANKL binding reduced breast epithelial stem/progenitor cell number. The stiff breast tissue from women with high mammographic density also had elevated RANK signaling and an expanded pool of stem/progenitor epithelial cells. The data link tissue fibrosis and elevated integrin mechanosignaling to stem/progenitor cell frequency and causally implicate hormone signaling in this phenotype. Thus we conclude that inhibiting RANK signaling would decrease the frequency of stem/progenitor cells in the breast to reduce the elevated lifetime risk to malignancy associated with high mammographic density and the aggressiveness of highly fibrotic breast tumors.

The mechanism of metastasis during breast cancer progression and how to inhibit it

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Multiphoton microscopy of live animals in real time led to the discovery of cause and effect relationships between cells leading to metastasis, relationships otherwise not possible to identify using *in vitro* models. This has revolutionized our understanding of cancer metastasis. Multiphoton imaging demonstrates that tumor cells migrate with macrophages and move with high persistence to blood vessels under the control of HGF gradients. At the blood vessel these migrating tumor cells interact with TMEM, the intravasation doorway, composed of a three cell complex involving the direct contact between a Mena-Hi tumor cell, endothelial cell and Tie2-Hi/VEGF-Hi macrophage. The TMEM structure itself, as well as the gene expression pattern of tumor cells interacting with TMEM, have been validated as prognostic markers for predicting metastasis in breast cancer patients. These were the first markers of metastasis in clinical use derived from multiphoton intravital imaging. TMEM doorways are the only sites in breast tumors where transendothelial migration of tumor cells and intravasation occur. TMEM doorways are found in both primary and metastatic tumor sites in breast cancer and in primary and metastatic sites of pancreatic ductal adenocarcinoma. Clinical trials of TMEM inhibitors targeting TMEM doorways in both primary and secondary sites, are now underway in breast cancer patients (clinical trials study number NCT02824575). As tumor cells interact with TMEM doorways, tumor cell crowding occurs around TMEM causing Mena INV expression in cancer cells in response to macrophage- induced NOTCH signaling, and other macrophage-dependent tumor cell programming associated with Cancer Stem Cells (CSCs), metastatic seeding and dormancy. Mena INV expression is necessary for transendothelial migration during intravasation at TMEM doorways and assures the efficient dissemination of seeding competent CSCs and hard to detect dormant tumor cells which, after a delay, contribute to metastatic growth.

The impact of disseminated cancer cell dormancy on the paradigm of metastasis

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Increasing evidence shows that cancer cells can disseminate from early-evolved primary lesions much earlier than the classical metastasis models predicted. It is thought that a state of early disseminated cancer cell (early DCC) dormancy can precede genetic maturation of DCCs and metastasis initiation. Here we reveal at single cell resolution a previously unrecognized role of mesenchymal- and pluripotency-like programs in coordinating early cancer cell spread and a long-lived dormancy program in early DCCs. Using *in vitro* and *in vivo* models of invasion and metastasis, single cell RNA sequencing and human sample analysis, we provide unprecedented insight into how early DCC heterogeneity and plasticity control the timing of reactivation. We identify in early lesions and early DCCs the transcription factor ZFP281 as an inducer of mesenchymal- and primed pluripotency-like programs, which is absent in advanced primary tumors and overt metastasis. ZFP281 not only controls the early spread of cancer cells but also locks early DCCs in a prolonged dormancy state by preventing the acquisition of an epithelial-like proliferative program and consequent metastasis outgrowth. Thus, ZFP281-driven dormancy of early DCCs may be a rate-limiting step in metastatic progression functioning as a first barrier that DCCs must overcome to then undergo genetic maturation.

Drug repurposing of hemostatic compound desmopressin (dDAVP) in triple-negative breast cancer (TNBC): preclinical antitumor activity on 2D/3D cell growth, chemotaxis, tumor progression and metastatic spread

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Desmopressin (dDAVP) is a repurposed hemostatic drug in oncology that acts as a selective agonist for the AVPR2 receptor present in blood microvessels and some tumor cells. Preclinical data show that compound triggers cytostatic mechanisms in malignant cells, impairing angiogenesis and metastatic progression. It is known that triple-negative breast cancer (TNBC) is associated to poor prognosis due to limited response to therapy and metastatic relapse. Considering the unsatisfied clinical needs of TNBC, we evaluated the antitumoral activity of dDAVP on aggressive preclinical models of TNBC, alone or in addition to chemotherapy. dDAVP significantly reduced clonogenic and 3D growth, viability and chemotaxis of AVPR2-expressing TNBC cells. Cytostatic effects of dDAVP were associated to altered actin cytoskeleton

dynamics and differential expression of migration, angiogenesis and metastasis-related genes. Synergistic effects were observed after combining dDAVP with taxane or alkylating therapy. In animals bearing TNBC tumors combined therapy resulted in greater inhibition of tumor progression, reduction of skin infiltration and metastatic spread to lungs. In conclusion, agonist activation of AVPR2 using dDAVP represents an achievable and interesting therapeutic approach to modulate TNBC aggressiveness. We propose dDAVP as a coadyuvant agent for treating this disease, not only in combination with chemotherapy but also administered during the perioperative setting.

Hypoxic microenvironment is associated with acquired resistance to HER2+ breast cancer immunotherapies
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Trastuzumab and trastuzumab emtansine (T-DM1) immunotherapies are the treatment of choice for HER2+ breast cancer patients. However, *de novo* or acquired resistance is still the main obstacle in clinical practice. To study the effect of hypoxia in acquired resistance, we used human mammary carcinoma BT-474 (HER2+) and MCF-7 (control) cell lines. As a hypoxia model, we added CoCl2 (100 µM) in cell culture medium. The hypoxic status of the cells was confirmed by a Western blot analysis showing a peak of HIF-1α expression after 6 hours. This result correlated with VEGF induction, as measured by RT-qPCR ($p < 0.05$). It is known that hypoxia has a role in regulating stem cell behaviour. Accordingly, BT-474 cells treated with CoCl2 developed a higher number of mammospheres than normoxic cells ($p < 0.01$). Then, we studied the hypoxia-mediated effect on trastuzumab and T-DM1 cell treatments. We found that BT-474 cells treated with increasing concentrations of the drugs for 72 hours presented a significantly higher viability under the hypoxic condition ($p < 0.05$), showing its cytoprotective effect. In MCF-7 cells, both drugs were less effective and no significant differences between conditions were found. In addition, a flow cytometry analysis showed that the drugs decreased membrane HER2 protein expression ($p < 0.01$) regardless of hypoxic microenvironment. Cellular mechanisms underlying the role of hypoxia in acquired resistance to HER2+ breast cancer immunotherapies are being studied.

Session 8 - Round Table 2 - Biorepositories and sample management
In Spanish at the end as a special article

Session 9 - Estrogen Receptors: Their involvement in endocrine Resistance and dormancy

ER mutations in breast cancer
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Constitutively active estrogen receptor-α (ER/ESR1) mutations have been identified in one third of ER+ metastatic breast cancer. Although these mutations are known mediators of endocrine resistance, their potential role in directly promoting metastatic disease has not yet been mechanistically addressed in greater detail. In this talk, I will present data supporting the idea that ESR1 mutations play critical roles in a number of metastatic processes, in an allele- and context-dependent manner.

Targeting dormancy in ER+ breast cancer
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Anti-cancer drug treatments often do not completely eradicate all cancer cells in the body, leaving behind dormant cancer cells that can ultimately develop drug resistance to give rise to recurrent disease. ER+/HER2- breast cancer is a disease in which dormancy is highly relevant: roughly 1/3 of patients (~400,000 women every year worldwide) with early-stage disease treated with surgical resection and adjuvant endocrine therapy eventually experience disease recurrence. Recurrences of ER+ breast cancer occur over a long time frame (> 20 years), suggesting that cancer cells can undergo prolonged periods of dormancy. Therapeutics that either maintain cancer cells in a dormant state or eradicate them would prevent cancer recurrence. We developed preclinical models of dormancy in ER+ breast cancer through estrogen deprivation, which occurs in patients treated with aromatase inhibitors as endocrine therapy. Such dormant breast cancer cells exhibit increased AMPK activation and mitochondrial respiration driven by fatty acid beta-oxidation. Pharmacological inhibition of beta-oxidation decreased dormant cancer cell burden in mice. However, pharmacological activation of AMPK or consumption of a high-fat diet blunted the antitumor effects of estrogen deprivation, leading to increased dormant BC cell burden. The metabolic features of dormant cancer cells may be exploited to develop improved treatments that would prevent disease recurrence.

Session 10 - Novel Targets in the Era of Precision Medicine

PARP inhibition in breast cancer
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Tumours with defective DNA repair by the homologous recombination repair (HRR) pathway are exquisitely sensitive to DNA damaging agents and to novel agents that block parallel pathways, including PARP inhibitors (PARPi). PARPi have been approved for the treatment of metastatic ovarian cancer (OvC) or breast cancer (BC). Currently used selection biomarkers to enrich the population of patients (pt) most likely to respond, namely the platinum-sensitive or BRCA1/2-mutated pts, have limited predictive capacity. There is a need for more specific biomarkers to guide personalized treatment. Genomic scar signatures have been proposed as a putative biomarker associated with DNA repair deficiency. A major limitation of these assays is the lack of specificity in HRR-altered tumours once they have restored the HRR function as mechanism of drug resistance. Instead, RAD51 foci formation is a functional and dynamic biomarker of HRR that correlates with PARPi response. We will review the current knowledge on PARPi sensitivity and resistance in breast cancer, response biomarkers and the potential of therapeutic combinations.

Use of CDK inhibitors in South America
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I will talk about the uses of CDK4/CDK6 inhibitors in South America. I will make a fast review of the contribution of CDK4/CDK6 inhibitors in advanced breast cancer, the discussions opened during last 2020 in the adjuvant setting, and then I will mention the current studies using these drugs and their availability in the real world.

News on PI3K inhibitors in clinical practice
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Abstract is not available

Antiproliferative effect of mifepristone in breast cancer patients with higher levels of progesterone receptor A than B: results from the MIPRA trial
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Preclinical data indicates that antiprogestins inhibit cell proliferation of luminal breast carcinomas expressing higher levels of progesterone receptor isoform A (PRA) than those of isoform B (PRB). MIPRA (NCT02651844) is an open-label, one-arm, prospective interventional study designed to test the effect of mifepristone (MFP; 200 mg, PO, QD, 14 days) in 20 breast cancer patients selected by their high PRA/PRB isoform ratio. The primary endpoint was to compare the Ki67 levels of the core needle biopsies and the post-therapy surgical specimens. Wilcoxon rank test was used to compare paired samples. A 49.62% decrease in the median was registered in all surgical specimens compared to baseline ($p = 0.0003$). Using the prespecified response parameter (30% reduction), we identified 14/20 (70%) responders. The degree of inhibition was similar to that reported for tamoxifen in luminal breast cancer patients in short-term treatment studies. RNA-seq analysis was performed in samples from 8 patients (4 responders and 4 non-responders) pre- and post-treatment. Interestingly, in responsive patients, MFP regulated genes related to the immune system and downregulated genes involved in cell-cycle and proliferative pathways. Our results show that MFP treatment may be effective in patients with a high PRA/PRB ratio. Ongoing analysis will determine changes in other markers that may help to further define MFP-responsive patients.

USP19 modulates cancer cell migration and invasion and acts as a novel prognostic marker in patients with early breast cancer
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The development of metastasis in patients suffering from cancer represents a significant reduction in their survival. Tumor cell migration and invasion are required for metastasis formation. In order to investigate the role of the Ubiquitin-Proteasome System (UPS) in the regulation of these processes, we performed a genetic screen using an shRNA library against UPS genes and Boyden chambers to analyze the migrating potential of breast cancer (BC) cells infected with this library. After the selection process, we characterized the non-migrating population and obtained a list of 30 candidate positive regulator genes. We focused on a specific DUB, USP19 and demonstrated that its silencing reduces the migratory/invasive potential of different BC cell lines. Since silenced cells proliferation was impaired using *in vitro* 3D setups, we furthered our investigation with *in vivo* studies. Mice inoculated with USP19 silenced cells presented Kaplan Meier curves for tumor free survival with a clear separation from the control group, as well as a delay in the onset of tumor formation. In addition, we observed a significant reduction in the generation of metastatic foci. Overexpression experiments in poorly migrating BC cells further validated our findings. Finally, we performed a retrospective clinical study which demonstrated that USP19 protein expression is a prognostic predictor of distant relapse free survival in BC patients. Altogether, USP19 might represent a novel therapeutic target.

Session 11 - Round Table 3 - Interaction among government, non-government agencies, and industry for funding and promoting breast cancer translational research
In Spanish at the end as a special article

Session 12 - Local and systemic therapies

Oligometastasis-The Impact of Local Therapy On Systemic Disease
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The development of systemic disease, or metastasis, has been long viewed as heralding the end of survival

from cancer. However, clinically, it has been observed for decades that some patients may harbor metastatic disease for long periods of time while other patients develop metastasis and succumb to disease rapidly, reflecting a spectrum of metastatic disease spread and virulence. Much of our ability to detect metastasis relies on advanced imaging techniques, which also have their limitations. Traditionally, treatment of metastasis with local therapies, (eg, surgery and radiation) were focused on palliation of pain or symptoms. In recent years, several trials have addressed the question of whether local ablative treatment of metastasis could improve outcomes in patients with limited metastatic disease, or oligometastasis. The treatment of oligometastasis with either surgery or ablative radiation resulted in improved long term disease control and survival in patients with several tumor types. This field is still evolving, and areas still being actively investigated include definitions of oligometastasis, advanced imaging techniques to identify subclinical metastasis and the underlying biology.

Treatment of HER2 Positive Tumors
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HER2 positive breast cancer is a very heterogeneous disease, clinically and biologically. Almost all breast cancer molecular subtypes are represented in tumors overexpressing HER2 inmunohistochemically. The treatment of early disease with neoadjuvant treatment is standard of care. We now know the importance of achieving pathologic complete response (pCR) and the changing characteristics of the residual disease. The use of double blockade with pertuzumab, trastuzumab and chemotherapy has augmented the proportion of patients with pCR. Another strategy is extended adjuvant treatment with neratinib in patients with residual disease ER+ or high risk ER+ disease. The use of Trastuzumab Emtansine in patients with residual disease has proven to improve results. Patients with low risk disease may de-escalate treatment, without the need of double blockade, less chemotherapy drugs and even shorter schemes. In the setting of advanced disease, many new treatments have emerged recently: Trastuzumab deruxtecan, Tucatinib, Neratinib and Margetuximab. Despite their different characteristics, all have shown responses in progressive disease after pertuzumab and trastuzumab and trastuzumab emtamsine. The challenge remains in the treatment sequence and the possibility to use them in early disease to cure more patients.

Immunotherapy for triple negative breast cancer
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Triple-negative breast cancer (TNBC) is an aggressive subtype of mammary carcinoma, which occurs in approximately 15% of diagnosed breast cancers. Thus, more effective therapeutic options are sorely needed. In recent years, advances in immunotherapy have yielded potential new therapeutic strategies which may be a viable option for the subset of immune activated TNBC. In early TNBC, pembrolizumab and atezolizumab have been tested in combination with neoadjuvant chemotherapy, resulting in higher complete pathologic response rates, regardless of PD-L1 status. Phase III clinical trials have shown a progression-free survival benefit with these two drugs in combination with chemotherapy in first line metastatic PD-L1 positive TNBC patients. These findings establish proof of principle for immunotherapy in both early and advanced TNBC. However, as efficacy is still low, we are in the need of developing more active immunotherapy combination regimens and more refined biomarkers that optimally identify patients most likely to benefit from immunotherapy.

MCL-1 is a clinically targetable vulnerability in breast cancer
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Pro-survival BCL-2 family protein MCL-1 is one of the most frequently amplified genes in breast cancer, particularly in 'basal-like' triple-negative breast cancer (TNBC). The outcome of most patients diagnosed with TNBC is especially poor, as post-surgical disease recurrence and mortality occurs in 50-80% of cases. The dismal prognosis of TNBC patients is caused by (1) increased propensity for metastasis; (2) high rates of post-treatment relapse and/or therapeutic resistance; (3) a paucity of targeted treatment options. We have previously demonstrated that MCL-1 protein expression in a large breast cancer tissue microarray cohort was predictive of poor outcome in treatment naive TNBC, thus representing an attractive therapeutic opportunity. We demonstrate that targeting MCL-1 restricts mammary tumour formation and promotes regression of established tumours *in vivo*. This is due to the canonical function of MCL-1 in the intrinsic apoptotic pathway where we uncover a requirement for MCL-1 in breast cancer stemness, and *in silico* analysis of breast cancer datasets highlights the correlative relationship between MCL-1 expression and markers of cancer stem-like cell behaviour. Targeted inhibitors of MCL-1 are currently undergoing clinical trials for the treatment of haematological malignancies, and our evidence suggests that targeting MCL-1 may offer a valuable new therapeutic option in TNBC patients.

Session 13 - New Developments in Diagnosis and Epidemiology of Breast Cancer

cfDNA analysis for breast cancer patients: fact checking
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No abstract available

Clinical and Molecular Landscape of Locally Invasive breast cancer in Latin American women
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Although gene expression-derived intrinsic subtypes were reported in Latin American breast cancer patients, most studies did not adequately represent the unique and diverse genetic admixture of our population. We assessed the general distribution and prognostic performance of

PAM50-based intrinsic and immunohistochemistry (IHC)-based surrogate subtype classifications in women included in a Breast Cancer Study initiative of the US-Latin America Cancer Research Network comprising institutions of Argentina, Brazil, Chile, Mexico and Uruguay. Eligible enrolled patients were characterized clinically, pathologically and epidemiologically and followed-up for 5 years. IHC subtypes were assessed according to St Gallen's 2013 criteria, using Ki67 to discriminate LumB from LumA tumors. A total of 1071 tumors were characterized by gene-expression microarrays. PAM50 classification defined 45% of tumors as LumA, 19.7% as LumB, 13.8% as HER2E and 17.5% as Basal. The 5-year prognostic ability of PAM50 and IHC classifications was also evaluated. PAM50-derived risks of recurrence (RORs), was used to discriminate risk into low, intermediate and high-risk groups. Transcriptomic pathway analysis was performed to identify the driven pathways in each subgroup and compared with TCGA stage matched samples. Current studies using molecular ancestry assignation may help to reveal subtler differences in this heterogeneously admixed population.

Mammographic breast density -the biological and clinical consequences of an opaque breast?

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Understanding Mammography (MBD) is crucial in the etiology and management of breast cancer (BCa). MBD is the expression of the fibroglandular: adipose content of the breast and is associated with the risk of developing BCa and the sensitivity of mammography (MMG). Although there are racial differences in the expression of MBD; the correlation of high MBD with an increased risk BCa is uniform. The common thinking is that high MBD is a normal variant; I would challenge this hypothesis. Without reproductive hormones high MBD does not occur. HRT usage can increase MBD and lead to an increased risk of developing BCa. Conversely when MBD is reduced, as with tamoxifen, the risk of developing BCa is reduced. Is this a causative change or just an association? Clearly, hormonal changes in the breast are critical in generating carcinogenesis. These changes start at a very early age in a woman's reproductive life and how we let the breast be exposed to these changes are critical if we are to alter the incidence of BCa. The following issues will be covered; • Measurement of MBD • Can high MBD be changed? • Can MBD be used as a surrogate marker in prevention trials? • What hormones are "good" for MBD? • How is MBD influenced by the hormone/immune interface? • Is MMG obsolete in high MBD?

A breast cancer patient-derived xenograft biobank for precision medicine studies in Argentina

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Patient-derived tumor xenografts (PDX) are generated by implanting tumor fragments directly from patients into immune-deficient mice. PDX reflect more accurately the human tumor biology as compared with cell line xenografts, since the latter have acquired different portraits due to long term culturing. Our aim was to establish a bank of genetically characterized breast cancer PDX models for precision medicine studies. The study was approved by Institutional IRB (021-2017). Surgical specimens from patients that attended "Magdalena V de Martinez Hospital" from General Pacheco were transplanted sc by trocar into estradiol (E2)-treated (0.5 mg pellets, sc) or untreated female NSG mice. Once the tumors reached 0.8 cm in diameter (long axis), they were excised, frozen, formalin-embedded for diagnosis, and passaged in E2-treated or untreated mice. A total of 95 samples were transplanted, and 9 PDX were developed: 2 luminal B, 1 luminal B that changed to triple negative (TN), 1 HER2 and 5 TN. Seven PDX were studied by Exome-Seq. Relevant mutations in FGFR1, ERBB2, STAT5A, FOXA1, BRCA1, PIK3CA, WNT4 and others were registered in the different PDXs. MUC2-19, HDAC6, HDAC7, Serpina2, LAIR1 among others were mutations present in almost all PDX. We conclude that these models are valid preclinical tools to test therapeutic efficacy of existing or novel drugs to guide breast cancer treatment.

Closing Conference

Quantitative Medicine for Breast Cancer Patients

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Gene-expression profiling has had a considerable impact on our understanding of breast cancer biology and clinical care. During the last 20 years, 5 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like and Normal-like), and a rare subtype with features of stem cells (Claudin-low), have been identified and intensively studied. The PAM50 subtyping assay provides important information within Hormone Receptor (HR)-positive breast cancer patients, where the Luminal A and B subtypes represent the majority of cases. Compared to Luminal A, Luminal B tumors are characterized by higher expression of proliferation/cell cycle-related genes and lower expression of several ER-regulated genes such as the progesterone receptor. The Luminal A vs B distinction, together with tumor size, is encompassed with the "PAM50 ROR Score", which quantitatively predicts recurrence, and thus can inform decision making concerning the length of endocrine therapy treatments (i.e. 5 years vs 10 years). In addition, genomic predictors of chemotherapy benefit for TNBC, and predictors of trastuzumab benefit for HER2+ patients, typically identify immune cell features as positive predictors of response, thus highlighting the importance of the microenvironment in response and survival. In particular, IgG/B-cell signatures were significantly associated with better metastasis-free survival. Overall, these data suggest that intrinsic molecular profiling, plus measures of the microenvironment focused on immune cells, can provide clinically relevant information beyond current pathology-based classifications.

POSTER SESSIONS

Poster Session 1

Chairs:
Juan Pablo Fededa, IIBio-CONICET, Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina
Vanesa Gottidredi, Instituto Leloir, Buenos Aires, Argentina
Estrella Levy, Centro de Investigaciones Oncológicas - Fundación Cáncer (FUCA), Buenos Aires, Argentina

PS1-1 / CDK4/6 inhibitors and antiprogestins: therapeutic combination in breast cancer experimental models with high levels of progesterone receptor isoform A
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Luminal breast cancers are susceptible to an endocrine therapy. Palbociclib (PALBO), a CDK 4/6 inhibitor, is currently used in combination with endocrine therapy to treat advanced hormone receptor-positive breast cancer (BC). However, with time patients acquire resistance. Therefore, alternative therapies are required to reduce BC mortality. We have recently reported that BC patients with tumors expressing higher levels of isoform A of the progesterone receptor (PRA) than isoform B (PRB) may benefit from an antiprogestin treatment. The aim of this study was to evaluate the effect of PALBO in combination with the antiprogestin mifepristone (MFP) in the T47D BC model. We have already shown that MFP (10 nM) inhibits cells proliferation of T47D and T47D-YA cells (expressing only PRA) but not of T47D-YB cells (expressing only PRB). PALBO (100 nM) inhibited cell proliferation in the three cell lines. The combination of MFP and PALBO induced an additive inhibitory effect only in T47D and T47D-YA cells ($p < 0.001$). To confirm these results *in vivo*, we inoculated T47D cells into NSG female mice. When the tumors were palpable, mice were treated with PALBO (20 mg/kg sc x 5 days a week) and/or MFP (0.5 mg/pellets; suboptimal dose), or vehicle. Only the drug combination was effective inducing a significant inhibition of tumor growth ($p < 0.01$) confirming the therapeutic potential of this combo. Ongoing studies will unravel the mechanism related with both pathways crosstalk.

PS1-2 / Parameters associated with metastatic dissemination are differentially modulated by specific isotypes of the retinoic acid receptor (RARs) in triple negative mammary cancer models
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Migration and adhesion are highly related to metastatic dissemination and retinoid system is implicated in their modulation. Objective: To evaluate the effect of activating each retinoic acid receptor (RAR) isotypes in migration, soluble MMPs activity, adhesion and metastatic potential in LM38LP and 4T1 triple negative murine cell lines. Both express all RAR isotypes except for RARβ in 4T1. Cells were treated with RARα agonist AM580 (200nM), RARβ agonist AC55649 (2μM), RARγ agonist BMS961 (50nM) or vehicle (DMSO). Migratory potential was evaluated by wound healing assay. AM580 and AC55649 reduced LM38LP migratory capacity ($p < 0.05$) while AM580 increased migration in 4T1 ($p < 0.05$). MMPs were analyzed by zymography. AM580, AC55649 and BMS961 decreased soluble MMP2 activity in LM38LP. On the contrary, AM580 increased MMP2 and MMP9 activity in 4T1. Besides, AM580 and AC55649 diminished LM38LP adhesive capacity while AC 55649 increased this in 4T1. In an experimental lung metastasis assay, cells treated with agonists where inoculated in BALB/c mice. The AC55649 pretreatment increased metastatic potential of LM38LP ($p < 0.05$) while BMSS961 increased metastasis in 4T1 cell line ($p < 0.05$). We hypothesize that the differences in RARβ expression between the cell lines could be responsible of opposite responses in biological effects studied. The increment in metastasis by RARβ/γ activation could be due to selection of a minority population with greater plasticity to colonize the metastatic site.

PS1-3 / Effect of 2'-nitroflavone on the expression of receptors associated with EGFR activity in breast cancer cells
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Flavonoids were proposed as chemopreventive and chemotherapeutic agents *per se* or in combination with traditional antitumoral drugs. Epidermal growth factor receptor (EGFR) is associated with tumorigenesis of several tissues and it can be involved in the molecular mechanism of action of several flavonoids. With the aim of investigating if a combinatory therapy involving 2'-nitroflavone (2'NF), a synthetic flavonoid with antitumor properties, and EGFR inhibitors would be a possible effective treatment for breast cancer, we studied if this flavone modulates the expression of EGFR or other receptors that interact or modulate EGFR activity. MDA-MB-231 and MCF-7 breast cancer cells were treated with 2´NF at different concentrations for 48 h. Afterwards, the protein content of EGFR, ErbB2, ER alpha, Met and IGF-IR were assessed by immunoblotting. Besides, PARP cleavage and phosphorylation of p38 were determined in the same experimental conditions. Results showed that 2´NF produced a reduction on the content of ErbB2 and IGF-IR in MDA-MB-231 cells, while in MCF-7 cells a decrease on the expression of EGFR, ErbB2 and ER alpha was observed. Both cell lines presented an increment in p38 phosphorylation and PARP cleavage upon 2'NF treatment. In conclusion, 2'NF demonstrated to have effects on the expression of receptors associated with EGFR activity which could justify a possible combinatory therapy involving 2'NF and EGFR inhibitors.

PS1-4 / Analysis of Akt molecular, subcellular and tumoral code as an explanatory and predictive tool for the effectiveness of therapies against breast cancer
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Cancer is a highly heterogeneous disease with significant cell-to-cell variability. Therefore, understanding the sources of this heterogeneity might help to design new therapies. Akt is a therapeutic target for cancer treatment and it is known to be regulated through numerous posttranslational modifications (PTMs) as well as to be recruited to different subcellular compartments. However, little is known about how a cell determines which substrates and functions Akt should regulate. Our hypothesis is that the Akt molecular code, *i.e.* the profile of PTMs of Akt, can determine its subcellular localization and vice versa, thus establishing the subset of Akt substrates and functions that Akt displays after each stimulus/cellular context. The aim of this study is to determine modification and subcellular localization patterns of Akt and its substrates in different mammary cell lines, both normal and tumor, and to analyze if a correlation between these variables and the resistance/sensitivity of these cell lines to antitumor drugs can be established. Using a strategy that combines automated imaging and quantitative measurement of Akt localization, we discovered novel Akt modifications and localizations. Preliminary results show that phosphorylation and localization patterns of Akt and its substrates differ between normal and tumor mammary cell lines. A bioinformatic study was performed to analyze association of Akt substrates grouped by cell compartment and different types of neoplasms.

PS1-5 / A potential strategy to prevent drug resistance: Chromosome instability can be prevented with no changes in the induction of cell death after Chk1 depletion
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The DNA damage response (DDR) is a complex network that assists the completion and fidelity of DNA replication upon DNA insults. Because cancer cells are subject to high levels of replication stress they heavily rely on the DDR. Conventional anticancer therapy exploits this vulnerability by inhibiting DDR effectors. Checkpoint Kinase 1 (Chk1) is a crucial mediator of the DDR whose inhibition is undergoing clinical evaluation, especially in prostate, ovarian and triple-negative breast cancers. It is currently accepted that DDR inhibitors trigger cell death as a consequence of increased replication stress and the ensuing chromosome instability (CIN). The link between replication stress and cell death has been validated in Chk1-deficient cell models; however, no unambiguous relationship has been established between replication stress, CIN, and cell death. Given that CIN fuels drug resistance, elucidating the molecular triggers of CIN and their relevance to cell survival is central to cancer research. We will present data, published in Calzetta et al., Sci. Adv., 2020, that unravel the identity of the molecular effectors of CIN activated by Chk1 deficiency. Unexpectedly, the pathway leading to CIN is independent of the one causing replication stress-dependent cell death. We propose that cancer treatment with Chk1 inhibitors might be improved by repressing the CIN pathway identified by us to avoid or reduce the generation of mutations that promote drug resistance.

PS1-6 / Exploring the role of hyaluronan and CD44 in resistance to ErbB-2-targeted therapies in breast cancer
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Hyaluronan (HA), through interaction with its receptor CD44, induces tumor progression. ErbB-2, a member of ErbB family of membrane receptor tyrosine kinases, migrates to the nucleus (NErbB2) where it acts as a transcription factor/coactivator to modulate proliferation and resistance to anti-ErbB-2 agents in breast cancer (BC). Accumulation of HA is associated with poor prognosis and promotes resistance to anti-ErbB-2 agent trastuzumab (TZ) in BC. Although crosstalk between HA/CD44 and ErbB-2 pathways has been reported, how their molecular interactions mediate TZ resistance remains unknown. Our *in silico* studies showed that TZ-resistant cells presented higher CD44 levels than TZ-sensitive ones. Stimulation with the ErbBs ligand heregulin (HRG) induces NErbB-2 translocation, acquired-TZ resistance and proliferation in SKBR3 cells. HRG also increased CD44 expression in SKBR3. In a *de novo* TZ-resistant BC model, JIMT1, the constitutive levels of nuclear CD44 (NCD44) and NErbB-2 were further enhanced by HA stimuli. Treatment with the chemical inhibitor of HA synthesis 4-methylumbelliferone (4MU) decreased not only HA levels but also NErbB-2 in JIMT1 cells. Furthermore, 4MU inhibited proliferation and migration of JIMT1 cells similarly to the inhibition observed when ErbB-2 was excluded from the nucleus via transfection with hErbB-2ΔNLS mutant. 4MU also inhibited HRG-induced proliferation in SKBR3. Our findings highlight the blockade of HA synthesis as a novel therapeutic strategy in TZ-resistant BC.

PS1-7 / GEF-H1 drives tumor formation, motility, invasion and metastasis in breast cancer
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RhoGTPases family are involved in several biological process including gene transcription, cell polarity,

migration and invasion. RhoGTPases switch between on and off states and they are regulated by several GEFs (activators) and GAPs/RhoGDIs (inactivators). The aim of this work is to study the role of a particular RhoA-GEF, GEF-H1, in breast cancer (BC) progression. We observed by immunostaining a significant increase of GEF-H1 protein expression in BC human biopsies compared with non-tumoral tissue. In addition, we observed that GEF-H1 expression correlates with the invasive potential of human and murine BC cell lines. To further study the role of GEF-H1 in tumor development, we generated GEF-H1-knock out (KO) BC cells using CRISPR/Cas9 technology. We observed a decreased in proliferation, migration, invasion and anchorage-independent colony formation in GEF-H1-KO cells *versus* wild type (WT) cells. These results correlate with a reduced focal adhesion formation and signalling. Furthermore, BALB/c mice were subcutaneously inoculated with GEF-H1 KO cells, showing a significant delay in tumor formation and lung metastasis development compared with WT cells. These results showed that GEF-H1-RhoA activation may mediate the signalling involved in controlling cell proliferation, migration and invasion of BC cells. *In vivo* assays and human biopsy analyses suggest that GEF-H1 expression in BC cell might indeed contribute to tumor progression.

PS1-8 / DNA polymerase iota prevents unleashed DNA elongation to promote cell survival and genomic stability

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The DNA damage response (DDR) is a multifaceted network of signals which is activated by structural and chemical alterations of DNA. The DDR involves DNA repair, DNA damage tolerance and checkpoint pathways which act in coordination to overcome genotoxic stress. It is unclear if all alternative DNA polymerases (Alt. Pols) have complete overlapped functions in DDR. By using siRNA technology we depleted the expression of 6 different Alt. Pols, either individually or in combination, and evaluated their relevant contribution to DDR after the exposure to DNA damaging agents. We analysed the induction of known replication stress markers such as the phosphorylation of H2AX (γH2AX) and the recruitment of 53BP1 to foci. From all of the Alt. Pols evaluated, only Alt. DNA polymerase iota (Pol ι) contributed differently to the induction of replication stress markers, were we observed reduced amount of γH2AX and less recruitment of 53BP1 to foci in the absence of Pol ι. We demonstrated that Pol ι prevents unleashed fork elongation, an unexpected result, as lack of Alt. DNA pols leads to reduced fork elongation which in turns promotes checkpoint activation and the accumulation of replication stress markers. In contrast lack of Pol iota leads to less checkpoint activation, increased cell death and genomic instability. All together our data suggests that Pol iota is implicated in the coordination of checkpoint signals that arise from elongating forks to promote the correct onset of DDR.

PS1-9 / Generation of a CRISPR/Cas9 EO771-tumor platform to study breast cancer cell - immune system interactions

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The main immunosuppressive mechanism by which cancer avoids eradication by the immune system is the expression of PD-L1, the ligand for T-cell inhibitory receptor PD-1. Despite their success in the treatment of different types of solid tumors, PD-1/PD-L1 blockade therapies have been ineffective in triple negative breast cancer (TNBC). Thus, it remains unclear which is the role of tumor PD-L1 and other immune checkpoint ligands in TNBC immune evasion. To address this, we developed a CRISPR/Cas9 expressing EO771 cell line as a platform to genetically study tumor-immune system interactions. As a first step, we confirmed the TNBC behavior of the EO771 model and characterized the immune response associated with the progression of EO771 tumor growth using flow cytometry. We found that EO771 tumor progression is hormone-independent and correlates with an increase in M2 macrophage polarization, a decrease in MHCII+ Antigen Presenting Cells (APCs), a marked increase in the CD4+/CD8+ ratio and CD4+ T cell exhaustion, which are consistent with tumor-mediated immunosuppression associated with poor prognosis in TNBC patients. In preliminary experiments, PD-L1 KO EO771 tumors show a significant but partial reduction in tumor growth rates, consistent with tumor PD-L1 being not sufficient to suppress anti-tumor immunity. By screening this platform, we will be able to massively test tumoral PD-L1 synthetic interactions to identify candidate genetic targets to overcome PD-1/PD-L1 resistance in TNBC.

PS1-10 / E3 UBIQUITIN LIGASE HERC1 REGULATES BREAST CANCER CELLS MIGRATION AND INVASION

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Tumor cell migration and invasion into adjacent tissues is the first step towards secondary tumors forma-

tion. Tumoral dissemination is associated with a significant reduction in patients' survival and life quality, and it is considered an unmet clinical need. In order to identify novel regulators of tumor cell migration/invasion within components of this ubiquitination pathway, we performed a pooled genetic screen using a shRNA library against ubiquitination-related genes and a highly invasive breast cancer cell line. We set up a protocol to specifically enrich positive migration regulator candidates that are involved *in vitro* and *in vivo* selection steps. Among the candidates we identified the E3 ligase HERC1. We demonstrated that its silencing reduces the migratory and invasive potential of breast cancer cells using *in vitro* experiments. We extended our investigation *in vivo* and confirmed that mice injected with HERC1 depleted cells display increased tumor-free survival, as well as a delay in the onset of the tumor formation and a significant reduction in the appearance of metastatic foci. Finally, we conducted an *in-silico* analysis and observed an inverse correlation between HERC1 expression levels and breast cancer patients' overall survival, suggesting that its overexpression could be a prognostic marker in patients with breast cancer. Altogether, our results highlight the potential of Herc1 as a novel putative therapeutic candidate for cancer treatment.

PS1-11 / Tristetraprolin (TTP) down-regulation in mammary epithelial cells induces developmental delay and DUSP-6 phosphatase overexpression

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Tristetraprolin (TTP) binds the 3'UTR of its target mRNAs, promoting their degradation. We have determined that reducing TTP expression levels in mammary cells increases expression of pro-inflammatory cytokines and apoptosis. Here, we show that TTP deletion in the mammary epithelium of MMTV-Cre/TTPfl/fl mice caused a delay in ductal elongation and the persistence of terminal end buds in the bi-transgenic adult females. Since we had observed that HC11 TTP knockdown cells (TTP-KD), displayed lower phosphorylated ERK1/2 (pERK) levels than HC11-shControl cells (Ctrl) while growing in complete medium, we proposed that sub-activation of this MAPK might be involved in the observed phenotype. Our results show that although the temporal sequence of ERK phosphorylation upon addition of EGF was not altered in TTP-KD respect to Ctrl, the first showed increased expression of DUSP6, an ERK1/2-specific phosphatase encoded by a TTP-target mRNA. Then, DUSP-6 overexpression may decrease pERK levels in proliferative conditions, without altering fast phosphorylation of this MAPK in response to growth factors. In summary, our results confirm the relevance of TTP in the mammary epithelium, suggesting that the activity of this protein on DUSP6 mRNA would be of vital importance for maintaining p-ERK1/2 high levels in proliferative conditions. More studies are underway to verify ERK1/2 and DUSP6 involvement in the MMTV-Cre/TTPfl/fl mouse phenotype.

PS1-12 / LIVER X RECEPTOR (LXR) activation may affect breast cancer risk through induction of tristetraprolin (TTP) in mamary epithelial cells

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Tristetraprolin (TTP), a protein coded by the Zfp36 gene, induces mRNA degradation of proteins involved in inflammation and tumorigenesis, while LXRa is a transcription factor that plays relevant roles in cholesterol and inflammation control. Our previous data show that both proteins are highly expressed and active in the mouse mammary epithelium during lactation. Besides, it has been proposed that a single nucleotide polymorphism, which may alter an LXR binding site in the human Zfp36 promoter region, is associated with lower TTP expression and worse prognosis in breast cancer patients. In order to analyze LXR role on TTP expression regulation in mammary cells, female mice were treated with the LXR agonist GW3965 (GW) or DMSO (control) by IP injection during 96h after weaning. Our results show that GW induced TTP, while inhibited IL-6, TNFa, LIF and S100A9 expression in the involuting glands. On the other hand, we analyzed the effects of GW as well as lactogenic hormones: glucocorticoids (Dex) and prolactin (Prl) on HC11 mouse mammary cells in culture. We found that both GW and Dex+Prl induced TTP and B-Casein mRNA expression. However, differently from the lactogenic hormones, GW did not trigger STAT5 phosphorylation. These results suggest that LXR may be involved in TTP expression regulation in the mammary gland through a STA5-independent mechanism. New experiments are underway to verify the impact of the LXR-TTP pathway on breast cancer progression.

PS1-13 / Morphological and histological studies in mammary glands of GH-overexpressing transgenic mice.

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Growth Hormone (GH) is central to stimulate growth, development and metabolism. It plays crucial roles in mammary gland development and growth, and its upregulation has been associated with breast cancer promotion and/or progression. To ascertain how high GH levels could promote mammary tissue oncogenic transformation, some molecular markers and morphological characteristics were analyzed in the mammary gland of virgin adult transgenic mice that overexpress GH. For this purpose, mammary gland whole mounts and histological studies were performed. In trans-

genic mice, whole mounting studies evidenced epithelial ductal elongation and enlarged ducts along with deficient branching and reduced number of lobules compared to control mice. The number of terminal end buds was similar in normal and transgenic breast tissue. Hematoxylin and eosin staining confirmed a reduction in the number of ducts and alveolar structures in transgenic mice, with a significant prevalence of ducts in detriment of lobules. However, in transgenic mice, a thickening in the ductal wall was observed. Immunostaining for ki67 and c-Fos expression were found to be increased in transgenic mice, while c-Jun was decreased and c-Myc showed no significant differences between normal and transgenic tissues. In conclusion, upregulation of GH induces morphological alterations in the mammary gland that affects its normal development. While these effects are non-tumorigenic per se, they might predispose to oncogenic transformation.

PS1-14 / Inhibition of SETD7 methyl-transferase activity impairs mammary epithelial cell differentiation and lactogenesis

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SETD7 is a lysine methyltransferase that targets many proteins with relevance to breast cancer and mammary epithelial biology such as ERα, E-cadherin, beta-catenin and STAT3. However, effects mediated by SETD7 in normal mammary epithelial cells (MEC) remain to be investigated. Our aim was to study SETD7 activity on cell proliferation, epithelial and lactogenic differentiation in two mouse MEC lines (HC11 and EpH4). HC11 and EpH4 were cultured to obtain functionally differentiated/lactogenic (DIF) cells. SETD7 catalytic inhibition was achieved using 8 nM of (R)-PFI-2 for 24h. Cell proliferation was evaluated by cell counting, epithelial cell differentiation by E-cadherin and beta-catenin levels and localization, lactogenic differentiation by qPCR of igfbp5, lactoferrin expression and by beta-casein levels, as well as TLC and GC-FID to characterize lipid composition. SETD7 mRNA and protein levels are induced upon lactogenic differentiation. In DIF cells, inhibition of SETD7 activity increased cell proliferation and downregulated E-cadherin and beta-catenin proteins as well as Lactoferrin, igfbp5 and beta-casein expression. Phospholipid metabolism related genes, Chpt1 and Pcyt2, were also affected by SETD7 inhibition resulting in altered lipidic profile different from the lipogenic phenotype of lactogenic cells. Altogether, the results suggest that inhibition of SETD7 catalytic activity impairs MECs lactogenic differentiation.

PS1-15 / Phenotypic and functional characterization of memory Natural Killer (NKm) cells in patients with Breast Cancer (BC)

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Memory-like NK (NKm) cells, present in individuals seropositive for cytomegalovirus (HCMV+), exhibit characteristics to be exploited in monoclonal antibody (MAb)-therapy directed against tumor antigens, mainly their amplified production of cytokines and proliferation in response to stimulation through CD16 receptors. We aimed to evaluate *in vitro* the functionality of NKm cells against Triple-Negative Breast Cancer (TNBC) cells opsonized with IgG1 isotype MAbs. Peripheral blood samples were obtained from healthy donors (HD; n = 16) and BC patients (n = 25) without prior treatment, at the time of primary tumor surgery. The proportion of individuals seropositive for HCMV+ was similar in BC patients and HD (84% vs. 81%, respectively). We determined two NK cell subpopulations based on their expression of FcεRIγ by FACS: FcεRIγ negative -NKγ⁻- (memory) and NKγ+ (conventional). BC patients presented a NKγ⁻ subpopulation in about half of the HCMV+ individuals with phenotypic characteristics similar to those previously described in these cells in HD: lower expression of NKp30 and CD161, and higher of NKG2C and CD85j, with respect to NKγ+ cells. Then, we performed functional assays. In HD, NKγ⁻ cells showed a higher production of IFN-γ ($p<0.05$; paired t-test) and TNF-α against TNBC cells opsonized with Cetuximab (anti EGFR) or Avelumab (anti PD-L1) than NKγ+ cells. Given that in some patients, NKγ⁻ cells were less functional than those in HD, this point will be further investigated after recruiting more patients.

PS1-16 / Study of Foxp3 expression in tumor microenvironment and peripheral blood of breast cancer patients

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Several clinical trials combining immune checkpoints inhibitors are in course both in breast cancer (BC) and in other tumors. In this sense, the study of the role of immune regulation in cancer is relevant. Foxp3 is known as a Treg marker, but also has been detected in tumor cells, being a controversial finding. The aim of this study was to evaluate Foxp3 expression in the tumor microenvironment and in peripheral blood mononuclear cells (PBMC) from BC patients. Tumor and peripheral blood from 127 breast cancer patients without treatment were obtained during surgery with informed consent. Foxp3 and other variables (ER, PR, HER2, CD8, TILs) were analyzed by IHC in tumors and by PCR in PBMC, and statistical analysis was performed considering also histopathological variables. *In silico* analysis of FOXP3 and coexpressed genes was performed through RNAseq and microarrays databases. Foxp3 was found in 61% of BC samples, showing a positive correlation with CD8+ cells and a negative association with tumor stage. In 73.5% PBMC samples, FOXP3 expression was found showing a positive association with advanced tumor stages and PR ($p < 0.05$). *In silico*

analysis showed that FOXP3 and coexpressed genes are associated to immune pathways and FOXP3 RNA levels were higher in Basal and Her2 subtypes. The presence of Foxp3 in breast cancer cells and FOXP3 expression in PBMC associated with advanced stages, makes this transcription factor a potential target for immunotherapy.

PS1-17 / Perforator flaps in the reconstruction of Breast and thorax burnts

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Oncological, traumatic and burn sequelae in the chest and breasts are problematic. The growth of tissues and sexual maturity, on scarred areas, produces glandular alterations, with the appearance of deforming skin bridles and contractures. Reconstructive surgery provides solutions to avoid deformities using neighbor perforator flaps. Eight patients (8-23 years) with severe sequelae in the thorax and breasts underwent surgery under general anesthesia using magnifying devices. Rhomboid design skin flaps and adipose tissue with a major axis interposed in the affected area were used. The arterial perforating vessel, centrally located, was rotated like a "helix" to reach a greater distance. Surgeries lasted 3 h and patients were hospitalized for other 24 h. We observed an average elongation of 5-7 cm in scars after surgery. All patients used for a year, compression elastic meshes. With this approach interposing healthy skin, an adequate development of the mammary region was achieved obtaining long lasting results with less morbidity, and faster recovery than traditional techniques. The complications were minor: partial dehiscences in the distal end. As a disadvantage, the skin of the armpit changed the hair orientation. In conclusion, we observed an improvement in the mammary tissue recovery in patients and greater mobility of the upper limbs using skin from healthy regions. In partial mastectomies this technique allows to reconstruct lateral defects, with stable long-term results.

Poster Session 2

Chairs:
Georgina Coló, INIBIBB-UNS-CONICET, Bahía Blanca, Buenos Aires Argentina
Luisa A Helguero, Institute of Biomedicine (iBiMED), University of Aveiro, Portugal
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PS2-18 / Aberrant Ret expression impacts on normal mammary gland post-lactation transition enhancing cancer potential

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Ret is a receptor tyrosine kinase with oncogenic potential in the mammary epithelium. Several receptors described as oncogenes in the breast have been shown to participate in specific developmental stages. We found that Ret is differentially expressed during mouse mammary gland development: Ret is present in lactation and its expression dramatically decreases in the following period of involution, the stage during which the lactating gland returns to a quiescent state after weaning. Based on epidemiological and pre-clinical findings involution has been described as a tumor promoting stage. Using the Ret/MTB doxycycline-inducible mouse transgenic system we show that sustained expression of Ret in the mammary epithelium during the post-lactation transition to involution is accompanied by defects in tissue remodeling and an enhancement of cancer potential. Following constitutive Ret expression we observed a significant increase in microneoplastic lesions in the post-involuting versus the virgin mammary glands. Furthermore, we show that abnormal Ret overexpression during lactation promotes factors that prime involution, including premature activation of Stat3 signaling and by RNA-seq an acute phase inflammatory response. Our results demonstrate that Ret contributes to a normal post-lactation transition and suggest that elevated Ret expression levels might be considered as a marker for postpartum breast cancer development.

PS2-19 / Tristetraprolin (TTP) Expression is Required for Mammary Progenitor Cell Survival

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Messenger RNA (mRNA) stability is regulated by proteins that bind to their 3´untranslated regions. One of them, Tristetraprolin (TTP), coded by Zfp36 gene, produces mRNA degradation of proteins involved in inflammation and tumorigenesis. We have previously proved that Wap-Cre x TTPfl/fl mice display apoptosis of mammary epithelial cells in which Zfp36 had been deleted during lactation. Here, we show that parity-induced mammary epithelial progenitor cells are particularly affected in those bi-transgenic females, since they display underdeveloped alveoli in their second lactation. Besides, multiparous females WAP-Cre x TTPfl/fl crossbred with RasG12D+/- mice presented fewer pre-neoplastic lesions than RasG12D+/- controls. Supporting the relevance of TTP expression in undifferentiated mammary cells, mRNA-seq data assessment indicates that progenitor populations display higher levels of Zfp36 than differentiated cells. Moreover, stem-like HC11 mammary cell line stably transfected with TTP-shRNA, exhibited decreased capacity to form mammospheres (MS) and to repopulate cleared fat pads. This phenotype was associated with high expression of pro-inflammatory cytokines as well as p38, NFkB, STAT3 and Caspase 3 activation. We also found that MS formation capacity was increased blocking TNFα or inhibiting p38 phosphorylation. In summary, our results indicate that TTP plays a relevant role in mammary progenitor cell survival, by keeping in line stress-associated pathways.

PS2-20 / Epigenetic changes induced by pesticide exposure reactivate LINE-1 retrotransposon in breast cancer and mammary epithelial cells

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Expression of long interspersed nuclear element-1 (LINE-1) is reactivated during breast cancer initiation and progression. Strong ligands of aryl hydrocarbon receptor (AhR) activate LINE-1 through the transforming growth factor-β1 (TGF-β1)/Smad pathway. Studies have linked breast cancer risk with pesticide exposure, including hexachlorobenzene (HCB) and chlorpyrifos (CPF), both weak AhR ligands which promote alterations in mammary gland and tumor growth in animal models. We examined the pesticides action on LINE-1 reactivation in MDA-MB-231 breast cancer cells and NMuMG epithelial breast cells, and we evaluated the role of TGF-β1 and AhR. Results show that 0.5 μM CPF and 0.005 μM HCB reduced the methylation of the 5´-UTR of LINE-1 and increased LINE-1 mRNA expression via Smad and AhR signaling in MDA-MB-231. Besides, 5 μM CPF and 0.005 μM HCB heighten ORF1p nuclear import, the protein encoded by LINE-1, through TGF-β1/Smad and stimulate DNA double-strand breaks. Disturbingly, 5 μM CPF and 0.005 μM HCB also enhanced LINE-1 mRNA levels in NMuMG cells. CPF effect was through AhR and TGF-β1, while HCB action depends only of AhR. In addition, both pesticides increased ORF1p expression and nuclear localization. In conclusion, HCB and CPF induce LINE-1 reactivation, not only in breast cancer cells but also in epithelial mammary cells, supporting the idea that pesticide exposure could promote epigenetic changes, contributing to cell transformation and tumorigenesis in breast cancer.

PS2-21 / Norcantharidine treatment inhibits *in vitro* parameters associated with tumor progression in triple negative breast cancer cell lines

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Triple negative breast cancer (TNBC) is a subgroup of very aggressive mammary cancer that do not express estrogen or progesterone receptors and neither overexpress the HER2 receptor. Norcantharidin (NCTD) inhibits the progression of several types of cancer however, the effect on breast cancer has not been studied yet. So, we have evaluated the effect of NCTD on human (HS578T) and murine (4T1) TNBC cell lines. NCTD induced an important antiproliferative effect, with an IC50 of 56 uM for HS578T and 35 uM for 4T1 cell lines. This antiproliferative effect was accompanied with the reduction in ERK activated levels (p-ERK) as well as an increase in the Sub-G0 cell cycle fraction, compatible with the presence of apoptotic cells. In both cell lines, NCTD reduced adhesive and migratory capacities ($p < 0.05$, Anova test) also displaying an important reduction in MMP-9 secreted activity. Although these parameters could have a direct implication in the malignant progression, clonogenic and *in vivo* assays showed an inverse behavior. In this regard, the pretreatment of 4T1 cells with NCTD induced an increase in the number of *in vitro* colonies and no effect could be detected in the amount of experimental lung metastatic nodes. Even though some results obtained are encouraging, we must seek the appropriated therapeutic strategy, probably combining with another drug, in order to allow and effective use of NCTD for the treatment of triple negative breast cancer.

PS2-22 / Dual galectin-8 and ALCAM silencing delays triple negative breast cancer progression

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Triple-negative breast cancer (TNBC) comprises 10-15% of breast tumors, and lacks targeted therapy. Galectin-8 (Gal-8) is a tandem-repeat type galectin involved in cell adhesion and migration, angiogenesis and tumor progression. Here, we studied the tumorigenic properties of Gal-8 and its ligand ALCAM/CD166 in TNBC. We silenced both Gal-8 and ALCAM in MDA-MB-231 cells with specific (MDA-shGal8 and MDA-shALCAM, respectively) or scrambled shRNA lentiviral particles (MDA-shControl). Interestingly, both MDA-shALCAM ($p < 0.01$) and double silenced MDA-shALCAM-shGal8 ($p < 0.001$) cells showed decreased proliferation and Bcl-2 down-regulation ($p < 0.001$) compared to control cells. Moreover, ALCAM-silenced cells showed impaired ability ($p < 0.001$) to form anchorage-dependent colonies and tumor spheres. Silencing of ALCAM decreased cell adhesion and migration onto Gal-8-coated surfaces in a glycan-dependent fashion. Remarkably, either Gal-8 or ALCAM silencing significantly disrupted ($p < 0.05$) cell-cell adhesion. *In vivo*, in a TNBC experimental model, at 56 days post-inoculum (pi), MDA-shGal8 ($p < 0.05$) and ALCAM-silenced ($p < 0.01$) cells generated smaller tumors than control cells. Notably, at day 98 pi, tumors generated by MDA-shALCAM-shGal8 cells were even smaller ($p < 0.05$) than those generated by MDA-shALCAM cells. In summary, dual knock-down of Gal-8 and ALCAM induced a pronounced delay on tumor

growth. Further studies are needed to elucidate Gal-8 and ALCAM mechanisms inducing TNBC.

PS2-23 / Combination therapy of paclitaxel with calcitriol analogues: a new therapeutic option for Triple Negative Breast Cancer

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Triple Negative Breast Cancer (TNBC) is a heterogeneous group of tumors with high mortality and poor prognosis. Therefore, new therapeutic strategies are needed. Previously, the non-hypercalcemic calcitriol analogues EM1 and UVB1 have demonstrated promising antitumoral effects in BC cells. Hence, the aim of this work was to evaluate the effects of the analogues combined with paclitaxel (PTX) on the viability and migration of TNBC cells. The results show that EM1 or UVB1 in combination with low concentrations of PTX display a greater reduction of the viability of 4T1- murine TNBC cells, with respect to control and with the monotherapy. The Combination Index (CI) values of Chou & Talalay method were 0.03 and 0.01 for EM1-PTX and UVB1–PTX combinations, respectively, indicating a synergistic effect. Additionally, these viability effects were lost when the Vitamin D Receptor (VDR) was silenced in the cells, suggesting that VDR is necessary for the antitumoral effects. Moreover, the cell cycle analysis of 4T1 cells treated with UVB1-PTX combination showed arrest in GO/G1 at 72 h followed by cell death at 120 h of treatment. Also, we found a synergistic effect by combining EM1 and PTX in MDA-MB-231- human TNBC cells (CI value: 0.0002). Finally, UVB1-PTX combination displayed antimigratory effects on 4T1 cell line. Altogether, these results suggest the potential use of these novel calcitriol analogues in combination with the conventional chemotherapy in TNBC treatment.

PS2-24 / Association between higher KI67 and higher proportion of effector T CD8 lymphocytes in HER2+ Breast Cancer patients

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HER-2-amplified (HER2+) breast cancer (BC) is characterized by the high expression of genes related to human epidermal growth factor receptor 2 (ERBB2/HER2). HER2+ BC patients are treated with neoadjuvant (NA) chemotherapy including anti-Her2 antibodies, trastuzumab (TRZ), and pertuzumab (PER). Considering that the adaptive immune profile in patients with HER2+ BC undergoing NA therapy could have an impact on the response to treatment, in order to find predictive markers of the response, we characterized different subpopulations of peripheral blood T lymphocytes of these patients. We analyzed by FACS T lymphocyte subpopulations that displayed markers related to exhaustion, activation, and memory from PBMC of 49 patients diagnosed before TRZ+PER therapy, and 23 healthy donors (HD). We also evaluated the association between immunological markers and clinical variables. The patients who presented a high tumor cell proliferation marker (Ki67 > 20), associated with chemotherapy response, showed differences in memory populations compared with those patients with a Ki67 < 20 and HD. In patients with KI67 > 20, a lower proportion of CD8 + CM T cells (central memory) was observed ($p = 0.0028$; 0.047) and a greater proportion of effector CD8 + T cells ($p = 0.0357$; 0.023), supporting the role of immunotherapy in treating a subset of HER2+ BC. The role of T cell memory subsets predictors of response to neoadjuvant chemotherapy in HER2+ BC should be further evaluated.

PS2-25 / Peripheral blood NK bright cells are augmented in breast cancer patients non-reaching pathologic complete response to antibody anti HER2+-based therapy

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HER2+ breast cancer (BC) patients are treated with neoadjuvant therapy with trastuzumab and pertuzumab, both anti-HER2 antibodies. In this context, NK cells could play a fundamental role due to their ability to perform ADCC and secrete cytokines such as IFN-γ. We were interested in characterizing peripheral blood (PB) NK cells in BC patients compared to those from healthy donors (HD). Also, we wanted to determine if the peripheral immune profile is associated with the response to treatment with anti-HER2 antibodies. To do this, we analyzed by FACS 10 NK cell receptors (CD57, NKp30, NKp44, CD16, NKG2A, CD25, NKG2C, CD16, PD-L1, and TIM-3) on PBMC of 49 patients diagnosed before trastuzumab+pertuzumab therapy and 23 HD. Patients presented a higher percentage of NK dim compared to HD (95% vs 91%, $p = 0.0022$), and also showed a higher proportion of NK PD-L1+cells (4.13% vs 1.8%, $p = 0.0069$). Despite the low number of patients non-reaching patho-

logic complete response (5/37), it was found that they present a higher concentration of NK bright cells in PB (16 cells/µl vs 10 cells/µl, p = 0.0161). Moreover, those patients with lymph node involvement (36/47) presented a higher percentage of NK cells than those without lymph node involvement (11.74% vs 8.57%, p = 0.026). These preliminary data exhibit several characteristics in HER2+ patients that could be associated with therapy response in the future. Currently, patient recruitment and functional tests continue.

PS2-26 / Establishment of two Breast Cancer Patient Derived Xenograft (PDX) lines and their respective treatment-resistant PDX variant

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Patient Derived Xenografts (PDX) are widely used in basic, translational and clinical research of cancer mainly because they conserve the original histopathology and chemo-sensitivity of the donor tumor, and they are stable across subsequent passages. In Argentina this tool is not extensively available, and most cancer research labs use cell culture for *in vitro* experiments. Here we present the establishment of two lines of breast cancer PDXs, both generated in Mendoza from donor patients of local public and private hospitals. The first PDX (called M1), lacks the expression of HER2, ER and PR, (triple negative), and shows a very high growth rate, needed to be passed every 3 weeks. In line with its aggressive phenotype, M1 PDX is not responding optimally to classical chemotherapy. The second PDX, (called H1), presents overexpression of HER2 receptor and lacks ER/PR. Unlike M1, H1 PDX has an intermediate growth kinetics, being passed every 2,5 months, and responds suitably to HER2- targeted therapy Trastuzumab (TZM). With the purpose of developing an animal model to study the mechanism of TZM resistance, we generated a variant of H1 that remains unresponsive to TZM, by treating the mice with increasing doses of TZM in every successive passage (15-20-25 mg/kg). The H1-TZM-resistant PDX does not respond to TZM at almost twice of the initial doses. We believe that the generation of this tool will potentiate the clinical and translational research not only in our group but in all the breast BC research groups in Argentina.

PS2-27 / RNA-seq Identifies Nuclear ErbB-2-Induced Transcriptome as a Key Driver for Triple Negative Breast Cancer Growth

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Triple negative breast cancer (TNBC) does not express estrogen and progesterone receptors, and lack membrane overexpression/gene amplification of ErbB-2, a tyrosine kinase receptor. TNBC is a heterogeneous disease presenting four molecular subtypes. Up to 78% of TN tumors in the clinic belong to the basal-like (BL) subtype. We found ErbB-2 in an unanticipated scenario: the nucleus of TNBC (NErbB-2). Our study on ERBB2 alternative splicing, using a PCR-sequencing approach combined with RNA interference, revealed that BL cells express the canonical ErbB-2, encoded by transcript 1, and the non-canonical isoform c, encoded by alternative transcript 3. Evicting both from the nucleus or silencing isoform c only, blocks TNBC growth. To explore whether isoform c growth-promoting effect is due to its functions as a transcriptional regulator, we performed RNA-seq in BL cells expressing only this isoform. We identified a set of genes differentially regulated in BL cells where we evicted isoform c from the nucleus, as compared to control cells. In the up-regulated group, we found enrichment of pro-apoptotic and tumor suppressor genes and in the down-regulated one, genes involved in proliferation and stemness. Furthermore, our clinical studies identified NErbB-2 as an independent predictor of shorter overall and disease-free survival in 99 TN primary tumors. Collectively, our findings reveal the potential of NErbB-2 isoforms as novel therapeutic targets and clinical biomarkers in TNBC.

PS2-28 / Working together for the family: HER oncogenes co-amplification in breast cancer

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HER2 overexpressing tumors represent 15-20% of invasive ductal breast carcinomas (IDC). Even though they are treated with the monoclonal antibody trastuzumab, 20% of primary and 70% of metastatic HER2 tumors develop resistance. HER2 belongs to a 4-member oncogene family (HER1-4), which present the capacity to compensate inhibition. We believe it is relevant to know the state of the whole family to predict response to treatment. Here we have designed a probe mix to detect the amplification of the 4 HER oncogenes. One hundred and eleven IDC (54 fresh frozen and 57 FFPE) were analyzed by MLPA, and HER2 determination was validated prospectively by FISH, IHC and CISH (Pearson r = 0.95; 0.59; 0.97 respectively, p < 0.0001). Positive correlation between CNV and expression was observed in wet and *in-silico* analyses for the 4 oncogenes (Spearman Rank test p < 0.05). Of the 111 included samples, 26.12% presented at least one HER amplified, of which 23.07% showed co-amplifications. In addition, we developed a protocol based on MLPA-ddPCR, which allows the detection of the tumor proportion of co-amplified HER. In this case, MLPA reactions were performed on single cells using Taqman probes, and then

analyzed by ddPCR. By this, we detected intra-tumor heterogeneity for HER co-amplifications. Here we present 2 tools based in MLPA that can identify the co-amplification level and the intra tumor heterogeneity of the 4 HER oncogenes, contributing to the precision medicine of breast cancer patients.

PS2-29 / Molecular cross-talk between ER and ID4 in breast cancer

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Inhibitor of differentiation (ID) 4, a member of the ID family, has been shown to act as a tumor suppressor and as an oncogene in breast cancer. Our group has investigated this apparent discordant information and has found evidence that ID4 acts as a tumor suppressor only in estrogen receptor ER+ tumors and as an oncogene only in ER- tumors. Here we focus on ID4's tumor suppressor role and further investigate why ID4 is aberrantly methylated exclusively in ER+ tumors. EZH2 is a histone methyltransferase involved in the tri-methylation of lysine 27 on histone 3 (H3K27me3) and also promotes DNA methylation via DNMT recruitment. In breast cancer EZH2 is overexpressed and downregulates the expression of tumor suppressor genes via increased promoter H3K27me3. Since ID4 is hyper-methylated in ER+ tumors and since EZH2 expression is induced by estradiol we hypothesize that estradiol induces ID4 methylation through EZH2. We performed siRNA (EZH2), immunofluorescence and chromatin immunoprecipitation (CHIP) experiments in MCF7 breast cancer cell lines. Our results show that EZH2 regulates ID4 expression as confirmed by siRNA experiments, that estrogen treatment increases EZH2 expression and CHIP experiments reveal that estrogen administration increases EZH2 and H3K27me3 marks on ID4 promoter. Taken together our results show for the first time that estradiol induces ID4 methylation trough EZH2 in breast cancer cell lines.

PS2-30 / Methods to monitor the relevance of M phase in the synthetic lethal potency of Polo-like Kinase 1 inhibitors

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Homologous recombination (HR) deficiency due to loss of BRCA function in cells leads to a propensity to the genesis of different types of cancer. Conversely, such HR deficiency is exploited to cause synthetic lethality (SL) or tumor-specific cell death in BRCA-deficient cancers. Such a synthetic lethality can be achieved by using drugs such as PARP inhibitors (PARPi) that prompt the accumulation of substrates for HR, e.g., DNA double-strand breaks (DSBs), which cannot be repaired in HR deficient cells. The trigger for SL in BRCA deficient cells treated with PARPi is intimately associated with acute DNA replication stress. In contrast, we have recently reported that BRCA1-deficient cells can be killed in a manner independent from such an S phase-associated stress. We found that inhibition of PLK1, an M-phase master kinase, causes SL in BRCA1 deficient models in a manner that does not augment parameters of DNA replication stress. Instead, BRCA1-deficient cells treated with PLK1i aggregate into multinucleated structures that suggest M phase's role in the SL triggered by PLKi. To get insight into such a DNA replication stress-independent SL mechanism, we will systematically monitor chromosome segregation, and other M phase parameters (multipolar mitoses, chromosome bridges, and lagging chromosomes resolution) will be discussed in depth during the presentation of results.

PS2-31 / Epigenetic modifications associated to breast cancer in metabolic syndrome-like disease mice models

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Metabolic syndrome (MS) is a proven risk factor for Breast Cancer (BCa). Previously, we found prominent epithelial lining in breast ducts of MS-like disease mice, obtained by chronically feeding animals with high fat diet (HFD). Our aim was to assess epigenetic alterations in breast ductal epithelium and tumors from HFD fed mice. H&E stains from breast tissues obtained from HFD fed Balb-c mice showed increased nuclei size and mitotic rate with presence of apoptotic bodies and nucleoli compared to control diet (CD) mice. Breast ductal epithelium from these mice were evaluated by immunohistochemistry (IHC) using antibodies against DNA methylation (5MC) and methylated histones (3MeH3-K4, -K9, -K27 and 2MeH3K36). We found no differences between groups by immunoreactive score. Nu/nu HFD or CD fed mice were inoculated with MDA-MB-231 cells. Xenografts showed no differences in methylated histones expression between groups, but HFD showed an increase of 5MC (IHC) and its enzyme DNMT1 (RT-qPCR) expressions. Analysis of multiple microarray datasets from patients (Oncomine) showed EZH2 and DNMT1 upregulated in BCa compared to normal breast tissue. Additionally, analysis of functional genomic datasets (UCSC Xena) revealed significantly increased (DNMT1, EZH2, SUV39H1, SUV39H2) or decreased (EZH1, SMYD1, SETD7) expression of methyltransferases in BCa tissue compared to normal tissue. We propose DNMT1 and EZH2 as potential biomarkers to further explore MS-associated BCa diagnosis.

PS2-32 / Epigenetics, bioelectricity and laterality of breast cancer

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In previous studies we found unexpectedly in patients that left-right (L-R) breast cancers (BC) differed in their methylation profiles (DM). We opened a new research line in which we hypothesize that, given the L-R environments of breast glands are non-identical: i. the bioelectric communication of the tumor with the L-R context differs,

and ii. epigenetics has a crucial role in these differences. Our results, so far, are promising. We found in-silico that the top genes with L-R DM were involved in development, embryogenesis, and neural differentiation. We confirmed the same processes, by developing a MDA-MB231-Nod Scid Gama xenograft model and compared L-R tumoral methylation patterns by RRBS. With focus on ion channels, we found that depolarizing channels were more methylated in R breast tumors. This suggested that R sided tumors had a more polarized state as compared to L tumors. We setup an in-vitro model to treat MDA-MB231 cells with L-R conditioned extracts from normal human mammary glands and measured Ca2+ and $\Delta\psi p$ with fluorescent probes. Cytometry assays confirmed bioelectric differences in the same direction: a more polarized state of right-treated cells. When deepening on epigenetic regulators, we found in-vitro a subtle increase of DNMT3 (de-novo methyltransferase) in left-treated cells, and confirmed it in-silico. Our studies support a non-explored epigenetic-bioelectric-laterality hypothesis for BC, which could serve as proof-of-principle for other bilateral tumors.

PS2-33 / Investigating the role of CBFB in breast cancer
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CBFβ is one of the top 17 most recurrently mutated genes in breast cancer. As a crucial transcriptional co-activator, CBFβ improves the DNA-binding affinity of RUNX proteins and therefore transcription of RUNX target genes. Since RUNX proteins have been previously shown to play context dependent roles in breast cancer and CBFβ is an essential regulator of these proteins, we questioned whether it has a phenotypic consequence in this disease setting. In silico analysis of TCGA data using cBioPortal highlighted how CBFβ undergoes varying alterations depending on the subtype of breast tumours. Interestingly in vivo experiments using a MMTV-PyMT;MMTV-Cre, Cbfβfl/fl mouse model of breast cancer, did not present any overt effects on tumorigenesis. This may be due to the mosaic nature of MMTV-Cre expression in PyMT driven tumours and as such we are testing whether Cbfβ is deleted in these tumours through western blotting alongside incorporating an RFP reporter gene for fluorescence imaging of tumours in vivo. Additionally, we have generated inducible-Cre tumour-derived cell lines (MMTV-PyMT;ROSA-CreERT2;Cbfβfl/fl) and conducted a range of biological assays to determine the effects of acutely removing CBFβ on tumorigenesis ex vivo. Preliminary results show reduced rates of growth in tumour cells lacking CBFβ. Together these results will provide an insight into the context dependent roles of CBFβ in different models of breast cancer.

PS2-34 / Analysis of RUNX-CBFβ as a relevant regulator of RSPO3 expression in breast cancer cells
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We have recently determined that R-spondin3 (RSPO3), a secreted protein that potentiates Wnt signaling pathway, is a key modulator of tumor progression and stem cell behavior in basal breast cancer. Previous reports suggested the potential involvement of the RUNX-CBFβ axis on RSPO3 expression in mammary tumor cells. These preliminary observations were confirmed by our results showing that small molecules able to inhibit CBFβ-RUNX interaction caused reduction of RSPO3 mRNA and protein levels in MDA-MB231 breast cancer cells. These treatments also induced inhibition of cell migration, ability that was recovered upon addition of recombinant RSPO3. To further explore the mechanisms underlying the control exerted by RUNX-CBFβ on RSPO3, we performed an in silico analysis of publicly available data from two RUNX1 CHIP-seq reports and an ATAC-seq study from human breast cell lines. We aligned the emerging data with the occurrences of the RUNX1 DNA-recognition-motif in the Rspo3 locus. This approach revealed a few putative RUNX1 binding sites. Among them, an intronic Rspo3 region that seems to be particularly active in triple negative (TN) breast cancer cells deserves special attention. In summary, our results show that RUNX-CBFβ transcriptional activity might affect TN mammary tumors by controlling RSPO3 expression levels. More experiments are being carried out to determine the mechanisms involved and the impact of this pathway on TN breast cancer behavior.

PS2-35 / RUNX2 overexpression generates endocrine resistance in human luminal breast cancer xenografts
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T47D and IBH6 cells that overexpress RUNX2 show high levels of FGFR2 and FGF2, supporting the hypothesis that FGF2 increases RUNX2 and, in turn, RUNX2 increases FGF2, maintaining a positive loop. However, in these models RUNX2 overexpression generates tumor resistance to FGFR inhibitor therapy and show a more aggressive phenotype compared with control tumors. T47D and IBH6 are luminal breast cancer cells that express ER and PR. Our goal is to explore the role of RUNX2 and its relationship with hormone receptors in BrCa. The aim of this work was to evaluate the effect of endocrine therapy in RUNX2 overexpressing tumors. RUNX2 and control cells (C, empty vector) were injected into the flank of NSG mice. Animals were treated for 3 weeks with an antiestrogen (Fulvestrant, FUL; 0.5 mg/week) or an antiprogestin (Mifepristone, MFP; 6 mg pellets). Control tumors showed a significant growth inhibition with the therapy (C-T47D $p < 0.0001$ C vs FUL and MFP; C-IBH6 $p < 0.0001$ C vs FUL), a lower Ki67 index (C-T47D: $p < 0.0001$ C vs FUL, $p < 0.05$ C vs MFP, C-IBH6 $p < 0.05$ C vs FUL) and higher stromal remodeling compared with untreated ones. In both models, RUNX2 tumors were resistant to endocrine therapy and all animals bearing RUNX2-T47D tumors developed lung metastasis. Our conclusion is that RUNX2 promotes BrCa progression and is a key player in

the acquisition of endocrine resistance. We emphasize the relevance of the development of RUNX2 inhibitors to use in combination with standard therapy for BrCa treatment.

Poster Session 3

Chairs:
Matías Blaustein, iB3-DFBMC-FCEyN-UBA and CONICET, Buenos Aires, Argentina
German Gil, CIQUIBIC CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.
Natalia Rubinstein, iB3-DFBMC-FCEyN-UBA and CONICET, Buenos Aires, Argentina

PS3-36 / Role of Thyroid Hormones in breast cancer resistance to chemotherapy
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Breast cancer (BC) is the leading cause of cancer death in women. Combination chemotherapy is one of the important adjuvant therapies for BC after surgery. The efficacy of drug treatment is often limited by tumor cell resistance. Many BC acquire multidrug resistance (MDR) by upregulating the level or activity of membrane proteins such as Pgp, which enable the exclusion of cytotoxic substances from the intracellular environment. Previously we demonstrated that Thyroid Hormones (TH) modulate chemotherapy response in T cell lymphoma (TCL). However little is known about the modulation of TH in the mechanisms that lead to chemotherapy resistance in BC cells. Bexarotene (Bex) is an oral retinoid-X-receptor agonist that is effective for the treatment in cutaneous TCL and there are ongoing clinical trials studying its role for BC treatment in combination with chemotherapeutic drugs. However thyroid dysfunction is recognized as an important side effect of Bex treatment, potentially manageable by TH administration. We found that Bex treatment reduces intracellular drug accumulation in MDA MB 231 cells, while TH revert this effect. Bioinformatics studies revealed alterations of genes significantly associated with drug response and genes involved in the TH signaling pathways in paclitaxel resistant-BC cells vs paclitaxel resistant-BC cells treated with Bex. These results point out the role of TH in BC resistance to chemotherapy and strengthen the importance to check thyroid status during BC therapy.

PS3-37 / ACE2 downregulation as a resistance mechanism to VEGFR-tyrosine kinase inhibitor (VEGFR-TKI) treatment in breast cancer therapy
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Absence of effective treatment and tumor resistance to current therapies for triple negative breast cancer (TNBC) are crucial factors. Central in the mechanism of resistance is overexpression/activation of tyrosine kinase receptor (RTK). Inhibitors targeting RTKs, including VEGFR, induce resistance and metastatic disease in some patients. Renin Angiotensin (Ang) system has been implicated in cancer progression through ACE1/AngII inducing angiogenesis and metastasis. Ang-(1-7), is generated from AngII by ACE2. Previously, we found that AngII promotes invasion by activating VEGFR signaling in TNBC and Ang-(1-7) counteracts undesirable actions of AngII. Besides, it has been demonstrated that VEGFR-TKI treatment of renal carcinoma decreased ACE2 expression, and combination treatment with VEGFR-TKI and Ang-(1-7) generated additive suppression of tumor growth and survival outcomes. Here, we found that treatment with Axitinib or Bevacizumab significantly reduced ACE2 expression in two metastatic TNBC-like breast cancer cell lines (MB-MDA231 & EO771). Interestingly, this treatment did not alter ACE2 expression in a non-metastatic cell line (T47D), suggesting that this is a mechanism operating in a more aggressive phenotype. In contrast, we found an increase in ACE1 expression in RTK-driven tumor model. We suggest that the balance of ACE1/ACE2 expression could serve as an indicator of tumor malignancy and therapy resistance and we propose a preclinical breast cancer model to target ACE2/Ang-(1-7) axis.

PS3-38 / Cytokinesis inhibition induces synthetic lethality and mitotic abnormalities in BRCA2-deficient cell lines
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BRCA2 is involved in homologous recombination, a pathway that repairs DNA double strand breaks, one of the most lethal DNA lesions. Hereditary and somatic loss of function mutations in BRCA2 correlate with highly invasive breast and ovarian cancers that do not respond well to chemotherapy and have a poor prognosis. As such, there is an urgent need for alternative therapies. To find novel therapeutic targets, we screened a kinase inhibitor library and found that inhibition of a key mitotic kinase kills BRCA2 cells. This kinase regulates cytokinesis during mitosis and few data exist regarding crosstalk with DNA repair. Using a clinically approved and specific kinase inhibitor, we validated our screen in multiple BRCA2 cell lines. In BRCA2 cells, the kinase inhibitor induced mitotic defects such as multinucleation, aberrant metaphases and chromosome bridges. Abnormal mitotic figures were often accompanied by multipolar spindle poles and supernumerary centrosomes. Interestingly, S phase was largely unaffected. Additionally, siRNA downregulation of the mitotic kinase yielded the same phenotypes as the inhibitor, showing that the kinase is a bona fide target in BRCA2 cells. Altogether, our data suggest that inhibiting cytokinesis in BRCA2 cells induces mitotic abnormalities and polyploidy which are the likely cause of cell death. Intriguingly, these phenotypes are different than what is observed with PARP inhibition (i.e. replication stress) suggesting a new Achilles heel for BRCA2 cells.

PS3-39 / Emerging role of AhR / c-Src signaling in breast cancer cell migration induced by tumor acidosis
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Acidosis is an important factor on tumor development, but little is known about activated mechanisms of action. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor which triggers non-genomic effects through c-Src. Considering that AhR/c-Src axis promotes breast cancer progression when is activated by ligand, this makes it a possible target to be induced by the acidic tumor microenvironment. Our aim was to study the effect of extracellular pH (pHe) 6.5 on AhR/c-Src axis and its correlation with cell migration and metalloproteases (MMP)-2 and 9 activities, using two breast cancer cell lines (MDA-MB-231 and LM3) and the mammary epithelial cells NMuMG. We found that acidosis induces c-Src phosphorylation only in breast cancer cells through AhR, since it was prevented by the AhR inhibitor 4,7-o-phenanthroline (PHE). In addition, the pHe 6.5 was blocked with PHE or the c-Src inhibitor PP2. Cytosolic pH (pHi) was measured in MDA-MB-231 cells treated with pHe 6.5, founding a reduction from 7.6 to 6.9. Amiloride is an inhibitor of the Na+/H+ exchange 1 protein that is known to reduce pHi. MDA-MB-231 treatment with amiloride enhances c-Src phosphorylation in an AhR-dependent manner, suggesting that the reduction in pHi could be involved in AhR/c-Src activation. Evidence suggests that acidosis induces a pro-migratory phenotype in breast cancer cells through AhR/c-Src signaling.

PS3-40 / Soluble tumor necrosis factor alpha fosters tumor growth, innate immune evasion and resistance to lapatinib in HER2-positive breast cancer
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Lapatinib (L) is a dual EGFR/HER2 tyrosine-kinase inhibitor used in HER2+ metastatic breast cancer (BC), but its clinical benefit is < 25%. We reported that soluble TNF (sTNF) induces trastuzumab (T) resistance by upregulating mucin 4 (MUC4), a transmembrane glycoprotein that shields T epitope on HER2, and that women with HER2+/MUC4+ BC have worse survival. Here, we studied the participation of sTNF and transmembrane TNF (tmTNF) in L resistance and the innate immune response (IIR) in JIMT-1, a T and L-resistant BC cell line. We used the dominant negative protein INB03 (DN) to selectively block sTNF and etanercept (E) to block both TNF isoforms. DN or E fail to inhibit tumor growth alone but, combined with L, tumor growth decreased in a 54% and 34% respectively ($p < 0.0001$ vs. IgG). L+DN exhibited a stronger anti-tumor effect than L+E ($p < 0.05$). Tumor-infiltrating immune cell analysis showed an increase in NK cell activation and degranulation and a decrease in myeloid-derived suppressor cells in L+E and L+DN treated tumors. Here, we show that TNF blockade overcomes L resistance and that TNF neutralization along with L treatment unleashes an anti-tumor IIR, suggesting MUC4 expression in patients with HER2+ BC as a potential biomarker of L resistance. Patients with HER2+/MUC4+ tumors undergoing L therapy would benefit from the addition of the selective sTNF inhibitor DN to overcome resistance, particularly patients with CNS metastasis since L and DN cross the blood brain barrier.

PS3-41 / Effect of Lapatinib and All-Trans Retinoic Acid (ATRA) combined treatment on mammary cancer stem cells derived from HER2 negative cell lines
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Cancer stem cells (CSC) are resistant to chemo and radiotherapies. To validate CSC as therapeutic targets in breast cancer, we analyzed the effect of Lapatinib (Lp, HER2 inhibitor), ATRA or the combined treatments, on growth, cell cycle distribution and metastatic capacity of primary mammospheres (CSC enriched cultures) from HER2 negative cell lines (4T1, MCF-7 and T47D). We determined by WB that HER2 is overexpressed only in CSC subpopulation of all cell lines analyzed. Primary mammospheres were treated for 96 h with Lp1μM for 4T1; 5μM for MCF-7, 2μM for T47D cells combined or not with ATRA 1μM. ATRA treatment alone or combined with Lp only significantly reduced 4T1 mammospheres diameters ($p<0.05$ Anova test) and signs of cell death were also observed. The combined treatment induced cell cycle arrest at G0/G1 phase after 48h of treatment in 4T1 mammospheres, analyzed by flow cytometry. However, this combination not significantly induces cell cycle arrest in MCF- 7 mammospheres. Finally, we performed an experimental lung metastasis assay in mice pretreated with ATRA and Lp and observed that combination reduced metastatic potential of 4T1 cells derived from mammospheres ($p<0.05$ Kruskal Wallis). In the present work we have demonstrated that the CSC component of HER2 negative cell lines overexpress such receptor. Moreover, Lp and ATRA combined treatment can successfully reduce mammospheres growth and metastatic potential in 4T1 experimental model.

PS3-42 / Influence of Structural Variants of the TRIC-CCT complex´s genes on patient survival and the main molecular pathways related to breast cancer
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Breast cancer (BC) represents a major health problem for thousands of women around the world. In Argentina, the incidence and mortality rates are the highest, compared to other types of invasive tumors in women. Cancer is a genetic disease where the function of critical genes in the regulation of growth and survival of cells is altered.

Several types of mutations have been studied in order to identify new prognostic markers or therapeutic targets. In this work, we have focused on structural variants, particularly the Copy Number Variation (CNV). CNVs are DNA fragments of 1Kb to 5Mb in length, that are present in a variable number of copies compared to a reference genome. The chaperone complex "TRiC-CCT" assists proteins to acquire their 3D conformation. Among its clients are actin and tubulin, as well as Cdc20, Cdh1, CCND1, among others. Previous studies have revealed that high expression of this complex is related to a worse prognosis in patients with BC. We propose to evaluate the effect of CNVs in TRiC-CCT genes on patient survival and on the main molecular pathways related to cancer. To achieve this objective, the R programming language was used. We obtained the expression, CNV, and clinical data of 1,094 women with BC, from "The Cancer Genome Atlas" (TCGA). As promising results, amplifications observed in three CCT-members (TCP1, CCT2, and CCT8) are associated with worse survival outcomes. Also, multiple molecular pathways related to cancer such as cell cycle, focal adhesions, among others, are altered in relation to patients who do not present alterations in these genes.

PS3-43 / *In silico* identification of ADRA2A-associated miRNA expression as biomarkers for disease-free survival in breast cancer subtypes

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Breast cancer (BC) is the most frequently diagnosed and leading cause of cancer death among women worldwide. We previously described that the expression of the α2A adrenoceptor (ADRA2A) is an independent good prognostic factor in luminal tumors. We interrogated in the public TCGA database the expression of miRNAs able to bind to the ADRA2A 3´UTR and we selected for further studies those which correlated with ADRA2A expression. The cutoff points for disease-free survival (DFS) were selected using the Evaluate Cutpoint. Survival analysis was performed by Kaplan-Meier and log-rank (Mantel–Cox). A high expression of ADRA2A was significantly associated with better DFS as previously shown. hsa-miR 23a-3p, 30a-5p, 30e-5p, 33a-5p, 33b-5p, 135b-5p and 138-5p correlated with ADRA2A expression. When they were evaluated for prognosis ability, a high expression of 135b-5p is significantly associated with DFS in the whole cohort and in basal-like tumors in particular. The opposite was found for 23a-3p. A high expression of 30e-5p was associated with better DFS in luminal A, while 33a-5p was associated with longer DFS in luminal tumors in general, and luminal B in particular. In fact, high 30e-5p and 33a-5p expression exhibited 100% DFS in these subtypes. 30e-5p was overexpressed in luminal A (as has already been described) and 23a-3p, 30e-5p, 33a-5p, 135b-5p and 138-5p in basal-like tumors. These miRNAs and ADRA2A might be selectively used as prognostic biomarkers in the different BC subtypes.

PS3-44 / Integrative single-cell transcriptomic analysis unveils alternative polyadenylation modulation in the mouse mammary gland

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The mammary gland (MG) is a highly dynamic organ which undergoes periods of expansion, differentiation and cell death in each reproductive cycle. Single cell RNA-seq (scRNA-seq) analyses have contributed to understand the cellular and transcriptional heterogeneity of this complex tissue. Alternative polyadenylation (APA) generates diverse mRNA isoforms, which contributes to transcriptome diversity and gene expression regulation by affecting mRNA stability, translation and intracellular localization. This study is based on publicly available data from 53,686 individual cells obtained during mammary post-natal development, from puberty to post-involution. The original data-sets correspond to three foundational reports that have explored the MG cell populations throughout development at single-cell level using 3′tag-based scRNA-seq. This feature of the sequencing protocol allowed us to analyze APA patterns in the mammary epithelial cells (MECs). Our results show relevant changes in gene families associated with mRNA processing, such as hnRNP, Eif and Srsf, in different mammary cell linages throughout the post-natal phases. Besides, APA modulation is also observed in key mRNAs for MG development and function, as Egfr1 and Prlr, which encode EGF receptor and Prolactin receptor, respectively In summary, this study reveals that APA may provide a new control layer on gene expression regulation in MECs throughout puberty and adulthood.

PS3-45 / Differentiation of 3T3-L1 preadipocytes into beige adipocytes regulate mammary tumor cells behavior

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We previously demonstrated a metabolic and phenotypic switch of 3T3-L1 white adipocytes into beige adipocytes (browning) when presented to soluble factors secreted by mammary epithelial cancer cells. Browning of adipose tissue is an important contribution to the hypermetabolic state of breast cancer. The factors and signaling pathways responsible for the browning process are unknown, as well as the role of beige adipocytes could have on the tumor. Thus, the aim of study was to obtain insight into the effect of epithelial cell-beige adipocyte communication on tumor progression.We characterized components present in conditioned media (CMs) from beige adipocytes (BA) or white adipocytes (WA) achieved upon differentiation of 3T3-L1 preadipocytes, and evaluated the effects of BA-CMs and WA-CMs on both adhesion and migration of tumor (LM3, 4T1 and MC4-L1) and non-tumor (NMuMG) mouse mammary epithelial cell lines. Tumor cell lines revealed lower cell adhesion ($p < 0.01$) and increased cell migration ($p < 0.05$) after incubation with BA- and WA-CMs vs Control-CMs. In addition, MC4-L1 and LM3 cells significantly increased their migration ($p < 0.05$) in the presence of beige adipocytes. These results suggest that beige adipocytes secrete soluble

factors that regulate the behavior of both tumor and non-tumor mouse mammary epithelial cells, favoring tumor progression.

PS3-46 / Soluble factors from the tumor-adipocyte interplay stimulate morphological and metabolic changes in 3T3-L1 adipocytes

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Adipocytes are one of the primary stromal cells in mammary tissue, considered to play an active role in the tumor microenvironment. The aim of this work was to evaluate the effects of factors derived from conditioned media from human breast cancer adipose tissue (TCM) or normal breast adipose tissue explants (NCM) on lipolytic and mitochondrial changes and the expression of adipocyte markers. Adipocytes 3T3-L1 exposed to TCM showed an increase in the number of lipid droplets (LDs) but with reduced area, with a signal of discontinuous intensity for Plin1, and a reduction in cell area. Adipocytes exposed to NCM increased LDs size, without affecting HSL and Plin1 subcellular localization. Adipocytes incubated with TCM showed a tendency to increase expression of lipolytic proteins (HSL and Plin1) with a significant decrease in triglyceride level. 90% of the adipocytes incubated with TCM showed fragmented mitochondria, whereas only about 60% of those exposed to NCM presented this change. The highest percentage of mitochondrial fragmentation per cell was more frequent in adipocytes treated with TCM vs NCM. Adipocytes exposed to TCM showed a tendency to a decrease in white adipocyte (Caveolin, ATGL) and adipogenic (PPAR gamma) markers, and to an increase of immature (Pref-1) and beige adipocyte (UCP1) markers. These findings suggest that peritumoral adipocytes secrete factors which may induce morphologic and metabolic changes on adipocytes, that could contribute to tumor progression.

PS3-47 / The Kinin B1 Receptor induces the expression and secretion of IL-10 and M-CSF from breast cancer cells, favoring the differentiation and migration of macrophages

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The tumor microenvironment consists of a strong interaction between various cell types and molecular factors such as cytokines, growth factors, and proteases, which are intended to favor tumor progression. Kinins such as Lys-des[Arg9] bradykinin (LDBK) are key modulators that exert their effects by stimulating the kinin B1 receptor (B1R). Among the cells that compose tumor microenvironment are macrophages, which in a *milieu* rich in IL-10 and/or monocyte colony-stimulating factor (M-CSF) can be differentiated in a protumoral, M2 phenotype. Our aim was to determine if the stimulation of B1R with LDBK induces the expression and/or secretion of IL-10 and M-CSF in breast cancer cells and if their conditioned media (CM) induces macrophage migration and/or differentiation. For this, cells were stimulated with LDBK and the protein expression and secretion of IL-10 and M-CSF, in cell extracts and CM was determined by protein microarray and/or western blotting. In addition, was determine that THP1 macrophages stimulated with CM from breast cancer cells boosted migration and expression of several differentiation markers. Our results demostrate that B1R activation in breast cancer cells induces the expression and/or secretion of IL-10 and M-CSF. Also, it could favor macrophage migratory capacity during tumor progression.

PS3-48 / Low-grade inflammation is related to KLK3 levels in breast cancer

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Kallikrein-related peptidases (KLKs) are serine proteases that have been considered as important biomarkers in cancer biology. It has been previously shown that KLK3, also known as a specific prostate antigen because it was initially found in the human prostate, is also expressed in human breast tissue. However, its role in breast cancer has not yet been clarified. Chronic low-grade inflammation (CLGI), defined as "the chronic production, but a low-grade state, of inflammatory factors" is a common route of several non-communicable diseases such as obesity and cancer. It is known that inflammation can increase the risk of some types of cancer, including breast cancer, which is the most commonly diagnosed cancer among women worldwide. CLGI is characterized by slightly elevated proinflammatory cytokines such as IL-1 beta and IL-8, among others, in systemic circulation, the levels of which are at 1000 times lower than those observed in acute inflammation. Our goal was to explore if the CLGI condition may modify the expression levels of KLK3 and KLK4. For that reason, we treated breast cancer cells with a low dose of cytokines, similar to those found in the sera of breast cancer patients, according to the initial and advanced stages of the disease. At date, our preliminary results show that a chronic proinflammatory state is associated to the release of high levels of KLK3 and KLK4 from estrogen-sensitive breast cancer cells.

PS3-49 / Identification of circulating miRNAs associated to Kaiso and triple negative breast cancer in mice model
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Breast cancer (BCa) is the leading cause of death by cancer in women worldwide. Novel biomarkers for diagnosis and prognosis are necessary to increase patients' survival. Previous studies showed that Kaiso (ZBTB33) was increased in several cancer tissues, including BCa. This transcription factor regulates different genes/pathways that increase BCa growth and metastasis. Nuclear Kaiso predicted poor survival in women of African heritage who had triple negative BCa (TNBC). Due that Kaiso binds to LC3 it was linked to the secretory autophagy pathway. The aim of this work was to investigate the role of Kaiso on TNBC xenograft secretion of circulating miRNAs outside the tumor. We inoculated NOD scid gamma (NSG) female mice with Kaiso depleted (shKaiso) MDA-MB-231 (TNBC) or control cell lines. After tumor growth, we sacrificed the mice to collect blood and tumor samples. shKaiso inoculated mice showed alteration of the levels of MDA-MB-231-derived circulating miRNAs related to BCa and Kaiso, including miR-125b-5p, -16-5p, -21-5p, 93-5p. We also found that several Kaiso target genes were modulated in BCa xenografts, such as CCND1, FOXA1, DNMT1 and GATA3. Moreover, the autophagy-related proteins LC3A/B showed a significant accumulation in cytoplasmatic sites of shKaiso inoculated mice in primary tumors and metastatic sites. These findings suggest that Kaiso might be involved in cellular secretion of miRNAs, which reinforce the role of Kaiso in autophagy.

PS3-50 / Potential use of PI3K pathway effectors and associated microRNAs as biomarkers of breast cancer prognosis
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PI3K/AKT/mTOR pathway is frequently altered in breast tumors and has consequently become a promising therapeutic target; therefore, novel biomarkers are in need. In luminal breast cancer cell lines, we have previously found that AKT isoforms play distinct roles in tumor progression, with AKT1 promoting cell proliferation and AKT2 favoring cell migration and invasion. To further investigate the potential use of these proteins as breast cancer progression biomarkers, we analyzed tumor histology and expression of AKT1, AKT2 and downstream S6 phosphorylation (pS6) by immunohistochemistry in 55 luminal breast cancer samples. Our results showed that AKT1 expression decreased with greater tumor stage and Nottingham score, while AKT2 and pS6 were associated with poor prognostic factors such as higher nuclear grade and Nottingham score. We next performed RT-qPCR to evaluate the tumor expression of AKT-associated microRNAs and found that miR-34a and miR-126 correlated positively with AKT1 and negatively with AKT2 expression. In sum, low AKT1 and high AKT2 and pS6 levels appear to be associated with poor prognostic markers in luminal breast tumors. Moreover, evaluating microRNAs that are differentially associated with AKT1 and AKT2 isoforms could potentially be an advantageous tool to assess tumor prognosis in patient blood samples.

PS3-51 / Glycerol-3P-Acyltransferase 2 expression in MDA-MB-231 cells modulates the expression of Long Non-Coding RNAs linked to survival prediction in breast cancer patients
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We have previously shown that glycerol-3P-acyltransferase 2 (GPAT2) is overexpressed in various human cancer cell lines, including breast cancer MDA-MB-231 cells. We also showed that GPAT2 knockdown decreases cell proliferation, anchorage-independent growth, migration, and tumorigenicity, and increases staurosporine-induced apoptosis. GPAT2 is highly expressed in undifferentiated human breast carcinomas showing a significant positive correlation with the histological grade. This gene is also able to modulate the expression level of several small non-coding RNAs, including piRNAs and miRNAs, among others. Here we report a new analysis of a microarray study to assess the impact of GPAT2 silencing on the expression level of long non-coding RNAs (LncRNAs) in MDA-MB-231 cells. After identification, selection, and annotation of differentially expressed LncRNAs in GPAT2-silenced cells versus control cells, we conducted a series of bioinformatics analyses that allowed us to identify three LncRNAs (LINC1085, CTD2066L21.3 and NOVA1.AS1) predictive of overall survival in patients with breast cancer. In addition, we were able to identify specific miRNAs associated with the selected LncRNAs, in order to establish an interaction between both ncRNA groups to be considered for further experimental approaches and eventually therapeutic purposes.

PS3-52 / Establishing histopathological criteria for breast cancer research
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Breast cancer is a heterogeneous disease with varied morphological appearance, molecular features, behavior and response to therapy. Though these varieties are well-established and rely on clinical and histopathological classification, research demands the continuous study of the ever-increasing knowledge on the mechanisms behind disease progression and associated risk factors. In this context, novel diagnostic and therapeutic techniques may be

developed through experimentation on animal models. This dynamic interplay between clinical practice and experimental oncology faces new challenges, especially when clinicians and biologists interact at the bench. Here, we present our experience in unifying analytical criteria to classify and interpret histopathological studies beyond current standards. In biopsies of human breast carcinomas and mouse tumor xenografts, we evaluated histological and nuclear indexes to establish tumor grade. We evaluated the extent of necrosis in growing and shrinking tumors after therapy and the characteristics of tumor edges, whether they are expansive or infiltrating. After immunohistological studies, we designed a score for particular proteins in the PI3K/AKT/mTOR and cyclin D1/Rb pathways considering the percentage of positive tumor cells and the intensity of the staining. In conclusion, we established new approaches considering the research interest and the standard convention criteria with a dynamic interaction between scientists and pathologists.

Poster Session 4

Chairs
Karen Blyth, University of Glasgow, Glasgow, UK
Gareth Owen, Pontificia Universidad Católica, Santiago, Chile
Carolina Schere Levy, IFIBYNE-UBA-CONICET, Buenos Aires, Argentina

PS4-53 / Exposure to endocrine disruptors induces proangiogenic factors in MDA-MB-231 human breast cancer cells
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Angiogenesis plays a role in local tumor growth and metastasis. Elevated levels of Hypoxia Inducible Factor-1α (HIF-1α) correlate with angiogenesis. HIF-1α induces gene expression like Cyclooxygenase-2 (COX-2), Nitric Oxide Synthase-2 (NOS-2) and Vascular Endothelial Growth Factor (VEGF). COX-2 and NOS-2 promote tumor angiogenesis. VEGF increases endothelial cells proliferation, survival and migration. Endocrine disruptors Hexachlorobenzene (HCB) and Chlorpyrifos (CPF) induce cell proliferation and tumor growth in breast cancer animal models. Our aim was to examine their action on breast cancer angiogenesis. We studied HCB (0.005, 0.05, 0.5 and 5 μM) or CPF (0.05, 0.5, 5 and 50 μM) effect in MDA-MB 231 triple negative breast cancer cells on: HIF-1α, COX-2 and NOS-2 protein levels, VEGF expression and secretion (Western Blot). Our results showed that exposure to 6 h of HCB enhances HIF-1α levels at 0.05, 0.5 and 5 μM, and NOS-2 expression and VEGF secretion at all assayed doses. In addition, at 24 h HCB (0.05 and 5 μM) increases COX-2 levels ($p < 0.05$). Moreover, CPF for 6 h enhances HIF-1α and NOS-2 expression at all assayed doses, as well as VEGF secretion at 0.05, 0.5 and 5 μM. Besides, CPF (0.05, 0.5 and 5 μM) stimulates COX-2 levels at 24 h ($p < 0.05$). We demonstrated that HCB and CPF induce the proangiogenic factors expression. In conclusion, these data highlight that the exposure to endocrine disruptors could contribute to mammary carcinogenesis, inducing angiogenesis proteins.

PS4-54 / Chlorpyrifos subthreshold exposure induces epithelial-mesenchymal transition in breast cancer cells
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Chlorpyrifos (CPF) is one of the most frequently used pesticide in extensive agriculture around the world. The effects of this pesticide on carcinogenesis are not clear and there is no consensus concerning the risks of this compound. In this study we investigate whether CPF promotes epithelial-mesenchymal transition (EMT) in breast cancer cells. Migration and invasion were evaluated by wound healing assay, Boyden Chamber assay and multicellular spheroids (3D). Also, we analyzed the effects of CPF on the number and the area of first (MS1) and second (MS2) generation of MCF-7-mammospheres. We demonstrate that 50 μM CFP induces invasion in MCF-7 and MDA-MB-231 cells (*** $p < 0.001$) when they were grown as a monolayer. In MCF-7-3D culture, we observed that 0.05 μM CPF increased the area of invasion after 7 days (** $p < 0.01$) and CPF at 50 μM increased the area of invasion after 5 (* $p < 0.05$) and 7 days (*** $p < 0.001$) when collagen type 1 was used as matrix model. When Matrigel® was used as substrate, we observed that only 0.05 μM CPF produced an increment of the invasion area after 2 (* $p < 0.05$), 5 (** $p < 0.01$) and 7 days (*** $p < 0.001$). In addition, 0.05 and 50 μM CPF increases migration in both cell lines grown as a monolayer and 3D culture. CPF at 0.05 μM induced an increment of the number and the area of MS1 and MS2 (* $p < 0.05$ and *** $p < 0.001$). Our results show that CPF promotes migration and invasion in breast cancer cells, generating a more aggressive phenotype.

PS4-55 / Environmental exposure and mammary tumors: the pesticides action on LM3 murine breast cancer cells
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Epidemiological studies have shown that pesticide exposure is associated with an increased risk of breast

cancer, while the organochlorines body burden may influence the development of a specific subtype of breast tumor. Human epidermal growth factor receptor 2 (HER2) is associated with poorer survival, while the role of the estrogen receptor β (ERβ) in breast cancer is not entirely clear. The organochlorine pesticide hexachlorobenzene (HCB) is a weak ligand for the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor related to vascular and tumor development. Stem cells are of great interest for their ability to originate, maintain and expand tumors, as well as to cause metastasis and recurrences. Our aim was to investigate the HCB action (0.005, 0.05, 0.5 and 5 μM) on the expression of proangiogenic factors, cell viability, proliferation, migration and mammospheres development in murine breast cancer cells LM3 (ERα-/HER2+). Our results indicated that HCB increased cell viability, proliferation and migration at 0.05 and 5 μM, through an AhR-dependent mechanism ($p < 0.001$). In addition, HCB induced the expression of VEGF and COX-2 at 0.05 and 0.5 μM ($p < 0.05$) and, in all the doses tested, the pesticide stimulated the development of mammospheres while reducing the ERβ expression ($p < 0.001$). These results suggest that HCB promotes a dedifferentiated, proliferative and proangiogenic environment, contributing to tumor progression in a HER2+ breast cancer model.

PS4-56 / Implications of TGF-β in the early progression of breast cancer

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The TGF-β pathway activates the EMT process and enhances the stemness of normal and cancerous breast cells. This suggests that TGF-β is implicated in the early stages of breast cancer progression. On this basis, we propose to understand the implication of TGF-β in the dialogue between the two populations that conform the mammary duct: luminal cells (LEP) and myoepithelial cells (MEP). The cellular mouse model LM38 is composed of LM38-LP (MEP and LEP), LM38-HP (LEP) and LM38-D2 (MEP). Previously, we showed that only LM38-LP was able to develop DCIS tumors after intraductal injections, suggesting that bi-cellular interaction could confer an advantage for tumor formation and progression. Moreover, treatment with conditioned medium of MEP induced viability on LM38-LP. LM38-D2 showed higher levels of TGF-β1 mRNA than LM38-LP (qPCR, $p < 0.05$). LM38 cells were treated with a recombinant TGF-β1 (1ng/μl) and an inhibitor of its receptor SB431542 (10 and 20μM). We could observe that TGF-β1 treatment increased 40 percent the viability of LM38-LP compared to the control, which is reduced in the presence of SB431542 (CV, $p < 0.05$, $p < 0.001$). LM38-D2 tumors showed higher expression levels on TGF-β1 than LM38-LP (IHQ, $p = 0.03$). Expression of TGF-β1 presented a heterogeneous pattern in LM38-LP tumors which requires further characterization. These results suggest that TGF-β1 is one of the contributing factors in the LEP-MEP dialogue and could be involved in the DCIS-IDC transition.

PS4-57 / Effect of Roscovitine and mifepristone on luminal breast cancer cell proliferation

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The use of CDK2 inhibitors, such as Roscovitine (ROSCO), appears as a therapeutic alternative to overcome the acquisition of resistance to Palbociclib in luminal breast cancer. The aim of this work was to evaluate the effect of ROSCO on cell proliferation in cells differing in their progesterone receptor (PR) isoform context. We have already demonstrated that T47D-YB human breast cancer cells (only expressing isoform B; PRB) had increased cyclin A levels compared to T47D-YA cells (only expressing isoform A; PRA). We decided to evaluate if cells overexpressing PRB would be more sensitive to CDK2 inhibitors than those overexpressing PRA. T47D, T47D-YA or T47D-YB cells were treated with FGF2 to increase cell proliferation and then they were treated with ROSCO 2 μM. All cells were similarly inhibited by ROSCO ($p < 0.01$) and this was accompanied by a decrease in the levels of phospho-ERK. When FGF2-treated cells were incubated with ROSCO 1 μM and/or the antiprogestin mifepristone (MFP; 10 nM), only in T47D-YA cells the combined treatment showed significant inhibitory effects on cell proliferation that were stronger than those induced by the single treatments ($p < 0.01$). These results suggest that the levels of cyclin A are not a biomarker of CDK2 inhibitor responsiveness. In addition, we show that CDK2 inhibitors may be an option for luminal breast cancer cells overexpressing PRA isoform in combination with antiprogestins.

PS4-58 / Isolation and Characterization of Extracellular Vesicles coming from Breast Cancer Cells and Macrophages

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Antiestrogenic adjuvant treatments are first-line therapies in patients with breast cancer positive for estrogen receptor (ER+). Improvement of their treatment strategies is needed because most patients eventually acquire endocrine resistance and many others are initially refractory to anti-estrogen treatments. The tumor microenvironment plays essential roles in cancer development and progress; however, the molecular mechanisms underlying such effects remain poorly understood. Breast cancer cell lines co-cultured with TNF-α conditioned macrophages were used as pro-inflammatory tumor microenvironment models. In our simulated pro-inflammatory tumor microenvironment, Tumor Associated Macrophages (TAMs) promoted proliferation, migration, invasiveness, and breast tumor growth of ER+ cells, rendering these estrogen-dependent breast cancer cells resistant to estrogen withdrawal and tamoxifen or ICI treatment. We proposed that extracellular vesicles (EVs) are one of the most important players in cellular communication and they could be one of the responsible of the endocrine resistance we had observed. Isolation and characterization of EVs is the first step to analyze the content of the vesicles in our cells of interest for further functional assays that will allow us to determine whether they are involved in the communication between TAMs and tumoral cells, and if EVs are responsible of the endocrine resistance that some ER+ cancers acquired with endocrine treatments.

PS4-59 / Protein aggregation and hormone-related cancers: a novel approach to improve diagnosis and overcome endocrine therapy resistance?
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Protein quality control (PQC) network-autophagy, proteasome and the unfolded protein response (UPR)-is triggered by stress and is overactive in acquired antiestrogen (AE) therapy resistance. We hypothesized that AE therapy induces accumulation of aggresomes in sensitive cells hindering the activation of survival pathways. AE-sensitive (MCF-7 and T-47D) or resistant (MCF-7R and T-47DR) breast cancer (BC) cells were treated with AE for 24 h. Insoluble proteins were analyzed by LC-MS/MS. RTCB expression was inhibited with siRNAs and its effects evaluated by cell counting, IF and WB. Aggresome load correlated with apoptosis and was increased in sensitive cells. LC-MS/MS analysis identified a set of proteins with essential function in PQC only in sensitive cells, among them the UPR modulator RTCB. Aggregation of RTCB induced by AE correlated with impaired XBP1s expression in sensitive cells. Knock down of RTCB was sufficient to restore sensitivity to tamoxifen in resistant cells and increased the formation of aggresomes, leading to apoptosis. Analysis of primary human BC and metastases appearing in the same patient after AE therapy showed that RTCB is only localized to aggresomes in the primary tumours. Different protein aggregation patterns indicate loss of function of essential proteins that can be used to identify AE-resistant BC cells and improve the response to therapy. This supports the idea that AE-resistant cells that originate metastasis have a higher capacity to preserve RTCB function and, consequently, to successfully induce UPR and autophagy to maintain low levels of protein aggregation.

PS4-60 / Evaluation of mycotherapeutic antitumoral potential of the *Pleurotus ostreatus* I-Fraction in breast cancer
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Mycotherapy is one of the most promising complementary and integrative approaches in cancer care. Mushrooms compounds have been shown to exert antitumoral activity stimulating the immune system and modulating signaling pathways or cellular capabilities aberrantly-activated in cancer. Previously, we demonstrated the antitumoral and antimetastatic activity of Grifola frondosa D-Fraction in breast cancer, in vitro and in vivo. Recently, we have also focused on edible mushrooms cultivated in Argentina. In this context, the purpose of the current study is to evaluate the antitumoral potential in breast cancer of Pleurotus ostreatus I-Fraction, an extract of water-soluble polysaccharides obtained from the fruiting body. We found that I-Fraction decreased the viability of 4T1 cells mammary adenocarcinoma (triple-negative, murine) in a concentration and time-dependent manner (24 and 48 h; $p < 0.001$). In addition, I-Fraction (2.5 mg/mL, 48 h) increased the number of 4T1 cells in the subG0/G1 phase ($p < 0.001$) and decreased those in the G0/G1 phase compared to vehicle ($p < 0.001$). These results suggest that I-Fraction decreases 4T1 cell viability through an induction in cell death. In addition, we found that I-Fraction decreased migratory ($p < 0.01$) and invasive ($p < 0.001$) capability of 4T1 cells at 13 h of treatment, compared to vehicle. In conclusion, these results demonstrate the mycotherapeutic antitumoral potential of P. ostreatus I-Fraction on 4T1 breast cancer cells.

PS4-61 / Investigating the tumour suppressor function of RUNX1 in breast cancer
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RUNX1 has previously been shown to have both tumour-suppressive and oncogenic roles, depending on the context. For example, RUNX1 function is commonly lost in various haematological malignancies but appears to act as a dominant oncogene in some subtypes of leukaemia. Similarly in breast cancer, the subtype appears to have a significant impact whereby ER-positive breast cancers often have RUNX1 mutations with associated loss of function, yet RUNX1 expression in the triple-negative (ER-/PR-/HER2-) subtype has been correlated with poor outcome in patients. We present definitive in vivo evidence of RUNX1 acting to restrict tumour development in two independent preclinical models of breast cancer (driven by PyMT and WNT signalling, respectively) in which conditional loss of Runx1 results in early tumour onset and increased tumour burden. We are currently exploring the mechanisms behind the tumour suppressive functions of RUNX1, with a particular focus on the transcriptional alterations that are initiated upon deleting this transcription factor complex. CRISPR/Cas9-mediated deletion of RUNX1 in the HC11 mammary cell line has revealed that loss of RUNX1 drives mammary cell stemness in mammosphere assays and colony forming

assays. Loss of RUNX1 function also appears to enhance the stemness-promoting effects of Wnt3a-treatment in the 3D context. Conversely, ectopic expression of RUNX1 in these cells decreases mammosphere and colony formation capabilities, and dampens Wnt-enhanced stemness.

PS4-62 / RUNX1 participation on TNBC

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Triple negative breast cancer (TNBC) is associated with epithelial-mesenchymal transition (EMT), which might be involved in tumor chemoresistance according to growing evidences. Our group has shown that RUNX1 could be involved in the aggressiveness of this subtype of breast tumor. We reported that RUNX1 is able to promote cell migration and regulate tumor gene expression, like RSPO3 and GJA1, EMT and metastasis-related genes. ChIP assays done in our lab revealed that RUNX1 can regulate transcription factors involved in EMT. We observe a significant upregulation of RUNX1 gene expression in TGFβ-treated murine tumor cell lines. Moreover, RUNX1 protein expression correlates with poor patient prognosis in human samples of TNBC. Our aim was to evaluate RUNX1 gene expression and participation in drug-treated TNBC cell lines. Here we show that RUNX1 and GJA1 gene expression is significantly upregulated in doxorubicin-treated MDA-MB-231. Interestingly, we observe that loss of RUNX1 transcriptional activity strongly enhance doxorubicin toxicity in this cell line. Furthermore, we found a potential DNA binding site for glucocorticoid receptor (GR) in RUNX1 gene. MDA-MB-(231, 453 and 468) cell lines show that RUNX1 mRNA is significantly upregulated with dexamethasone (GR agonist) and downregulated with mifepristone (GR antagonist). Therefore, our data suggests that RUNX1 may be involved in TNBC chemoresistance and its expression could be externally regulated by GR activity modulation.

PS4-63 / Regulation of RUNX1 by androgen receptor, a new potencial mechanism of TNBC progression

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Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype for which no effective targeted therapies are available. Growing evidence suggests that chemotherapy-resistant cancer cells with stem-like properties (CSC) may repopulate the tumor. Therefore, therapies that target the CSC in combination with chemotherapy might prevent tumor recurrence. Androgen Receptor (AR) is expressed in at least half of all TNBC. AR inhibition decreases CSC in vitro and tumor initiation in vivo. RUNX1 correlates with poor prognosis in TNBC patients. Our group has shown that RUNX1 promotes TNBC cell migration and regulates tumor gene expression, like RSPO3 and GJA1. Our goal is to investigate the role of RUNX1 in the TNBC-RA+. Here we show that RUNX1 mRNA and protein expression is upregulated by treatment with DHT (AR agonist) in MDA-MB-453 cells, and that this effect is blocked in the presence of Enzalutamide (AR antagonist). ChIP-seq experiments revealed AR binding to RUNX1 regulatory regions in MDA-MB-453 cells, suggesting a direct regulation. Also, RUNX1 inhibition by AI-10-104 (a synthetic drug) produced a reduction in MDA-MB-453 and BT-549 cell proliferation and an enhancement in treatment sensibility. It has been reported that AR inhibition combined with chemotherapy result in more effective than chemotherapy alone in vitro and in vivo. In line with this, RUNX1 inhibition could be a potential target to also potentiates the anti-tumor effect of AR inhibition.

PS4-64 / AR and ErbB-2 Interaction in Triple Negative Breast Cancer

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We propose the existence of an interaction between androgen receptor (AR) and ErbB-2 which is involved in NErbB-2+/AR+ BC growth. The experimental model used was the human TNBC cell line MDA-MB-453 which displays high expression levels of AR and NErbB-2. By Western Blot we found that dihydrotestosterone (DHT) treatment for short periods of time (minutes) led to an increase in ErbB-2 phosphorylation at Tyr877 which we have proved to be required for ErbB-2 nuclear migration. By Immunofluorescence and subcellular fractionation studies we demonstrated that DHT induced ErbB-2 nuclear migration. By chIP we found that DHT induced ErbB-2 recruitment to a HAS site in FKBP5, a classical AR responsive gene. Finally, by microarray we identified 315 differentially expressed genes in the presence of DHT and NErbB-2 eviction by transfection with an ErbB-2 mutant which is unable to translocate to the nucleus and functions as a dominant negative inhibitor of ErbB-2 nuclear migration (hErbB-2ΔNLS). Multivariate Cox regression analysis identified the combined expression of 6 genes (CXCL10, TAP1, STAT1, NMI, HLA-A and NLRC5) as an independent predictor of better clinical outcome in TNBC. In conclusion, our findings evidenced that DHT-activated AR induces ErbB-2 rapid activation and its migration to the nucleus where it binds to HAS sites in the DNA. Moreover, based on the diffetentially expressed genes of NErbB-2 eviction in presence of DHT we identified a gene signature associated with favorable outcome in TNBC.

PS4-65 / Targeting androgen receptor and WNT pathway in endocrine-resistant mammary carcinomas with high AR and low ER and PR levels

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Endocrine resistance remains a major drawback in the treatment of luminal breast cancer (BC). The mechanisms that contribute to hormone resistance may include deregulation of hormone receptors and growth factor signaling pathways. We previously demonstrated that overexpression of fibroblast growth factor (FGF2) in endocrine responsive T47D cell line, induced tumor progression. To explore the mechanisms underlying endocrine resistance we performed RNAseq analysis that revealed a deregulated WNT signaling pathway in T47D-FGF2-overexpressing cells compared with control T47D cells. We also detected decreased estrogen receptor α (ER) and progesterone receptor (PR) along with an increase in androgen receptor (AR) expression, both at mRNA and protein levels. Hence, FGF2-overexpressing cells have higher AR/ER and AR/PR ratios than control cells. Thus, we tested the effect of targeting the AR and/or WNT signaling pathways on cell proliferation and tumor growth. In endocrine resistant cells, dihydrotestosterone (DHT; AR agonist) induced cell proliferation while the combined treatment with enzalutamide (AR antagonist) and LGK974 (WNT inhibitor) inhibited tumor growth and reduced the number of large metastasis. Conversely, DHT inhibited control T47D cell proliferation and AR blockage had no significant effects on tumor growth. Our results suggest that targeting AR and/or WNT pathways may be a therapeutic alternative for endocrine resistant BC with high AR and low ER and PR levels.

PS4-66 / Low Oct4 expression in mesenchymal stem cells contributes to the development of the bone marrow pre-metastatic niche in advanced breast cancer patients

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The imbalance between osteogenesis and osteoclastogenesis in the bone marrow (BM) microenvironment seems to play an essential role in the establishment of bone metastasis in untreated advanced breast cancer patients (BCP). We have previously found that this lack of balance is produced, among other factors, by a lower self-renewal, proliferation, and osteogenic differentiation capacity of BM-mesenchymal stem cells (MSCs). Mechanisms mediating these characteristic changes remain elusive. Here, we evaluated the expression of the osteoprogenitor marker CD146 (Flow cytometry), telomerase activity (qPCR), telomere length (qPCR), as well as the expression of the pluripotency factors Oct4 and Sox2 (qPCR) in BM-MSCs from clinical stage IIIb BCP (n = 8) vs. healthy volunteers (HV; n = 8). We found that MSCs from BCP had lower percentage of CD146+ cells ($p = 0.04$), decreased CD146 relative fluorescence index ($p = 0.002$), lower telomerase activity ($p = 0.04$), and shortened telomere length ($p = 0.002$) compared with HV. Moreover, Oct4 and Sox2 expression decreased by 54% ($p = 0.03$) and 72% ($p = 0.009$) in BCP-MSCs, respectively. Interestingly, Oct4 silencing impaired the ability of BM-MSCs to differentiate into osteoblasts ($p < 0.0001$). In conclusion, we found that a low Oct4 expression characterizes the altered BM-MSC phenotype in BCP. This change may explain the loss of osteoprogenitors and the impairment of MSC osteogenic processes, which create an ideal environment for BM metastatic development.

PS4-67 / CIN-independent cell death in S phase induced by pol eta depletion

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Defects in DDR lead to genomic instability that may trigger tumorigenesis. Genomic instability can manifest as a higher rate of acquisition of gross numerical or structural changes in the chromosomes, known as chromosomal instability (CIN). CIN is associated with the development of resistance to treatments, it is clear that it is recurrently elevated after DDR components' inhibition. That is why, although it is challenging, to think of strategies that induce cell death without generating abrupt and acute changes in CIN levels. When evaluating the mechanisms of cell death after the elimination of a DNA polymerase (pol eta), we found that the depletion of pol exacerbates the cell death caused by DNA damaging agents without causing a concomitant increase in CIN. This response happens because, in the absence of pol eta, cells cannot complete DNA replication and are more efficiently arrested in S phase. Soon after the DNA damaging challenge, cells depleted from pol eta display augmented DSBs that persist over time. DSBs are followed by the accumulation of massive regions of ssDNA and pan-nuclear phosphorylation of histone H2AX, which has been shown to correlate with a commitment to cell death. We also found evidence of RPA exhaustion, a marker that characterizes cell death in S phase. Such results suggest that the modulation of specific DDR effectors could selectively promote cell death in S phase, preventing CIN augmentation, a concept that may be relevant in clinical settings.

PS4-68 / Unveiling the mechanism of regulation of excessive fork elongation by Polymerase Iota

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To preserve the integrity of genetic information, cells must count with mechanisms-englobed by the term DNA Damage Response (DDR)-to protect cellular fitness when

DNA lesions accumulate during DNA replication. DDR includes processes of tolerance to damaged DNA, like Translesion DNA Synthesis (TLS), in which alternative polymerases (Alt. Pols) release replication stalling by using damaged DNA as replication templates. We found that one Alt. Polymerase, Pol Iota, has a novel function not shared with other Alt. pols in preventing unleashed DNA elongation. In doing so, Pol iota promotes the correct onset of checkpoint signals, preventing cell death and genomic instability. How Pol iota achieves this replication effect is unknown. Pol Iota has at least three associated functions: 1) it participates promoting the bypass of DNA lesions during TLS events; 2) its lyase motif allows it to repair abasic sites in Basic Excision Repair; 3) it interacts with the tumor suppressor p53 to reduce fork elongation speed. We were able to demonstrate that TLS and DNA repair were not required but p53 was required to control defective activation of checkpoint signals. Transient expression of exogenous Pol Iota rescued the DDR defects observed in CRISPR Pol Iota KO clones. In the future, we aim to identify the domain(s) of Pol Iota required for this non-canonical role of the Alt Pol.

PS4-69 / Effect of olaparib in combination with doxorubicin on MDA-MB-231 breast cancer cell line

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Breast cancer (BC) is the leading cause of cancer death in women in Argentina. Anthracycline-based regimens represent the major chemotherapeutic agents used in BC treatment. However, chemotherapy resistance development and adverse effects are still a challenge for the oncology. Poly [ADP-ribose] polymerase (PARP) is a key DNA repair protein mainly involved in base excision repair. In the last years, PARP inhibitors (PARPi) have been approved for the treatment of BRCA-mutated BC. As PARP is involved in doxorubicin-induced cellular response, the aim of this work was to determine the effect of the PARPi olaparib on the sensitivity of BC cells to the anthracycline doxorubicin. MDA-MB-231 cells were treated with increasing concentrations of doxorubicin (5-40 nM), olaparib (1 μM) or doxorubicin and olaparib during 24 h. Clonogenic assay, viability assay and immunofluorescence were performed. Simultaneous administration of the drugs resulted in a combination index <1, which indicates a synergistic effect of doxorubicin and olaparib. In addition, the cell viability was significantly lower for cells exposed to combined treatment in MDA-MB-231 cell line. Residual DNA damage (evaluated by γH2AX foci) was higher for cells exposed to simultaneous treatment with respect to individual administration of the drugs. These preliminary results suggest that PARP inhibition may potentiate the effect of doxorubicin in BRCA-proficient breast cancer cells.

PS4-70 / Expression of a fusion peptide that mimics the epitope of the HER2 antigen and its application for trastuzumab detection

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Trastuzumab (Herceptin®, Genentech Inc), is a humanized recombinant monoclonal antibody (mAb) directed against the extracellular domain of HER2/neu. Routine laboratory analysis of trastuzumab binding usually requires the expression of the HER2/neu antigen, which is a 185 kDa transmembrane glycoprotein, in mammalian cells. Because of its size and significant complexity, it is a difficult antigen to develop and produce. However, if we focus on the epitope, a peptide that mimics the binding site of trastuzumab can be designed instead of the complete protein. This would allow the use of a prokaryote expression system which has several advantages in productivity and costs. Here we show the development of a peptide that mimics the trastuzumab binding site on HER2/neu, a “mimotope” that can be expressed in Escherichia coli. We designed the vector with the sequence of the 13-residues peptide fused to Maltose Binding Protein (MBP), generated resistant clones and successfully induced the expression of the protein in a soluble form. Finally, we purified the fusion protein and verified by ELISA that can bind trastuzumab, but not other mAbs with different specificities. It is expected that this recombinant protein could be a useful tool for quantification of functional trastuzumab for development, quality control and clinical studies.

MESAS REDONDAS - ROUND TABLES

Mesa redonda 1 (Sesión 5): Plataformas genómicas

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Resumen

El cáncer de mama es una compleja enfermedad con características y curso clínico heterogéneos. En las últimas dos décadas han emergido ensayos de perfil de expresión genética como herramientas de pronóstico para ayudar en la toma de decisiones clínicas en el cáncer de mama y son el tema de esta sesión. Dos de estas pla-

taformas genómicas que se comercializan en Argentina son Oncotype DX y MammaPrint. Aunque difieren en las plataformas tecnológicas empleadas (PCR cuantitativa *vs.* Microarray), el número de genes (21 vs. 70) y en los genes específicos empleados, ambas plataformas han sido validados en su poder predictivo para pacientes con cáncer de mama temprano. En esta sesión, el doctor Ernesto Korbenfeld mostró los datos de predicción de la plataforma Oncotype DX en el marco del estudio clínico Fase-III TAILOR X para predecir recurrencia a distancia en pacientes con carcinoma de mama temprano. También presentó los resultados del estudio RxPONDER, para tratar de de-escalar el tratamiento de quimioterapia en estos pacientes. Por su parte, el doctor Fernando Petracci presentó la validación y utilidad clínica de dos plataformas de análisis combinado: MammaPrint/BluePrint que permiten subclasificar a los pacientes con cáncer de mama temprano RH + / HER2 - en 4 categorías de riesgo de metástasis: ultra-bajo riesgo, bajo riesgo, alto riesgo y ultra-alto riesgo. También incluyó datos del estudio Fase-III MINDACT donde se utilizó MammaPrint para evaluar el riesgo de metástasis a distancia en pacientes con cáncer de mama temprano y su potencial para identificar pacientes que no necesitarían la aplicación de quimioterapia.

Abstract

Round Table 1 (Session 5): Genomics platforms. Breast cancer is a complex disease with heterogeneous characteristics and clinical course. In the last two decades, gene expression profiling assays have emerged as prognostic tools to aid clinical decision-making in breast cancer and are the subject of this session. Two of these genomic platforms that are commercialized in Argentina are Oncotype DX and MammaPrint. Although they differ in the technological platforms used (quantitative PCR vs. Microarray), the number of genes (21 vs. 70) and the specific genes used, both platforms have been validated in their predictive power for patients with early breast cancer. In this session, Dr. Ernesto Korbenfeld showed the prediction data of the Oncotype DX platform in the framework of the Phase III TAILOR X clinical study to predict distant recurrence in patients with early breast carcinoma. He also presented the results of the RxPONDER study, to try to de-escalate chemotherapy treatment in these patients. For his part, Dr. Fernando Petracci presented the validation and clinical utility of two combined analysis platforms: MammaPrint/BluePrint that allow subclassifying patients with early RH + / HER2 - breast cancer into 4 metastasis risk categories: ultra-low risk, low risk, high risk and ultra-high risk. He also included data from the Phase III MINDACT study where MammaPrint was used to assess the risk of distant metastasis in patients with early breast cancer and its potential to identify patients who would not need chemotherapy.

Esta sesión de plataformas genómicas fue presidida por el Dr. Aníbal Núñez de Pierro del Hospital Fernández, Buenos Aires; y copresidida por el Dr. Ignacio Mc Lean del Hospital Universitario Austral, Pilar y el Dr. Gustavo Helguera del IBYME-CONICET, Buenos Aires, Argentina. El objetivo de esta sesión fue mostrar la aplicación de firmas de expresión génica en el cáncer de mama para la toma de decisiones informadas en la atención clínica.

La primera presentación estuvo a cargo del Dr. Ernesto Korbenfeld, jefe de la unidad de cáncer de mama del Hospital Británico de Buenos Aires. El Dr. Korbenfeld presentó la plataforma genómica Oncotype DX para la evaluación pronóstica y predictiva del cáncer de mama en pacientes con Receptores Hormonales (RH) + / HER2 - en enfermedad temprana. Mostró los datos de predicción de esta plataforma en el marco del estudio clínico TAILOR X que incluyó una población de 6.711 pacientes con carcinoma de mama temprano con RH + / HER2 - y con ganglios axilares negativos. También presentó los resultados de Oncotype DX en el contexto del estudio RxPONDER, el cual se diseñó con el objetivo de tratar de de-escalar el tratamiento de quimioterapia en pacientes con cáncer de mama temprano RH + / HER2 - y con uno a tres ganglios axilares positivos.

La plataforma Oncotype DX permite la medición de la expresión de un total de 21 genes, de los cuales 16 genes son de pronósticos / predictivos (vinculados a la expresión de los RH, del receptor HER2 y genes de proliferación e invasión) y cinco genes son de referencia. La determinación se realiza a nivel de ARN mensajero mediante PCR cuantitativo por transcriptasa reversa en tejido tumoral de mama fijado con formalina e incluido en parafina. Basado en los niveles de expresión relativa de estos 16 genes usando como referencia la expresión promedio de los 5 genes de control, se desarrolló una puntuación de recurrencia (*Recurrence Score*, RS) que predice el riesgo de recurrencia de la enfermedad a distancia a los 10 años para pacientes con cáncer de mama RH + / HER2 - y con ganglios linfáticos negativos tratadas con tamoxifeno adyuvante (Estudio NSABP-14)[1]. El RS, como variable continua de 0 a 100, se utilizó para estratificar a los pacientes recién diagnosticados con cáncer de mama invasivo temprano en 3 grupos de resultados de riesgo diferentes: un subgrupo de riesgo bajo de recurrencia (RS < 18), otro de riesgo intermedio de recurrencia (RS de 18 a 31) y el tercero de alto riesgo de recurrencia (RS > 31). El análisis de resultados mostró que la formación de metástasis a distancia a los 10 años de seguimiento fue del 6,8% en el grupo de bajo riesgo, 14,3% en el grupo de riesgo intermedio y 30,5% en el grupo de alto riesgo[1].

El Dr. Korbenfeld mostró datos del ensayo TAILORx, en el cual se evaluó el valor pronóstico y predictivo del Oncotype DX con un RS intermedio en un estudio prospectivo[2]. En este estudio clínico, pacientes de cáncer de mama con ganglios linfáticos axilares negativos, ER + y HER2 - con una RS ≤ 10 recibieron terapia endocrina sola, aquellas con una RS > 25 fueron tratadas con quimioterapia seguido de la terapia endócrina, mientras que aquellas con un RS intermedio de 11 a 25 fueron asignados al azar para recibir terapia endocrina con o sin quimioterapia. De los 10.273 pacientes elegibles en el ensayo, 1.126 (16%) exhibieron una RS < 11. Después de un período de seguimiento de 9 años para este grupo de bajo riesgo, se encontró que el 93,8% estaban libres de enfermedad invasiva y el 97% estaban libres de recurrencia en un sitio distante, mientras que la supervivencia general fue del 98,0%. Este estudio permitió establecer que pacientes con RS de 0 a 10 presentarían una tasa de recurrencia a distancia muy baja (2-3%) a 9 años recibiendo sólo terapia endócrina. También que en pacientes con un RS intermedio de 11 a 25 la terapia endócrina sola no era inferior a la terapia endócrina más quimioterapia en supervivencia libre de enfermedad invasiva (objetivo primario del estudio) e intervalo libre de enfermedad a distancia. Sin embargo, un pequeño beneficio fue observado en las pacientes ≤ 50 años con RS entre 16 a 20 (beneficio absoluto de 1,6% a favor de la quimioterapia adyuvante en sobrevida libre de

recurrencia a distancia) y entre las pacientes con un RS entre 21 a 25 (beneficio absoluto del 6,5% a favor de la quimioterapia). Las pacientes con RS alto entre 26 y 100 a pesar de recibir quimioterapia seguido de hormonoterapia tuvieron una recurrencia a distancia del 13% a los 9 años de seguimiento.

A continuación, el Dr. Korbenfeld presentó los primeros resultados del ensayo RxPONDER. El estudio consiste en un ensayo clínico aleatorizado de Fase III de terapia endocrina adyuvante estándar con y sin quimioterapia en pacientes de cáncer de mama con uno a tres ganglios linfáticos axilares positivos, RH + y HER2 - evaluados con la plataforma Oncotype DX y con RS < 25[3]. Mujeres mayores de 18 años con RS < 25 fueron aleatorizadas para recibir quimioterapia con terapia endócrina (rama control) o sólo terapia endócrina (rama investigacional) en una aleatorización 1: 1 utilizando tres factores de estratificación: (a) RS (0 a 13 versus 14 a 25); (b) estado menopáusico; y (c) disección ganglionar axilar versus biopsia del ganglio centinela. El objetivo principal fue determinar el efecto de la quimioterapia sobre la supervivencia libre de enfermedad invasiva (IDFS) y si el efecto dependía del RS. Los objetivos secundarios incluyen la supervivencia general, supervivencia libre de enfermedad a distancia, supervivencia libre de enfermedad local y toxicidad. De las 9.383 mujeres registradas se aleatorizaron 5.083 pacientes (54,2%). Con una mediana de seguimiento de 5,1 años, se han observado 447 episodios de IDFS. En pacientes posmenopáusicas con uno a tres ganglios linfáticos positivos con RS entre 0 y 25 (N = 3.350, 67%), no se observaron diferencias significativas en IDFS entre quimioterapia con terapia endócrina vs. terapia endócrina sola, lo que indica que no se obtienen beneficios de la quimioterapia adyuvante en este importante subgrupo. En pacientes premenopáusicas con ganglios linfáticos positivos con RS entre 0 y 25 (N = 1.665, 33%), se observaron diferencias significativas a favor de la rama secuencial de quimioterapia y terapia hormonal (5,2% en IDFS a 5 años), lo que indica un beneficio de la quimioterapia. El estudio RxPONDER en este momento muestra que la terapia adyuvante puede reducirse a terapia endócrina sola en pacientes posmenopáusicas con RS < 25. Sin embargo, existe un beneficio en IDFS y sobrevida global en pacientes premenopáusicas (en parte debido a la acción ablativa de la quimioterapia sobre la función ovárica) de la quimioterapia adyuvante.

El segundo orador de esta sesión fue el Dr. Fernando Petracci, médico oncólogo especialista en cáncer de mama, *staff* del Departamento de Cáncer de Mama del Instituto Alexander Fleming de Buenos Aires. El Dr. Petracci presentó la validación y utilidad clínica de dos plataformas de análisis combinado: MammaPrint (70-gene signature, 70-GS) / BluePrint (80-gene signature, 80-GS). MammaPrint es una prueba de diagnóstico molecular tipo *microarrays* basándose en la expresión combinada del ARN de 70 genes; subclasifica a los pacientes con cáncer de mama temprano RH + / HER2 - en 4 categorías de riesgo de metástasis: ultra-bajo riesgo, bajo riesgo, alto riesgo y ultra-alto riesgo. Mammaprint se desarrolló a partir de interrogar ~25.000 genes que representan el genoma humano completo para identificar en biopsias de pacientes de cáncer de mama una firma de expresión génica de 70 genes que fuera fuertemente predictiva del potencial de metástasis a distancia a 5 años y 10 años[4]. BluePrint es una firma genómica complementaria de MammaPrint, basada en *microarrays* que utiliza la expresión combinada de 80 genes para subclasificar los tumores de mama en subtipos moleculares tipo Luminal A, Luminal B, HER2 y subtipo Basal.

El Dr. Petracci presentó resultados del ensayo MINDACT, un estudio internacional de fase III, prospectivo, aleatorizado, donde se evaluó el riesgo de 6.693 pacientes con cáncer de mama en etapas tempranas con ganglios negativos comparando métodos que emplean factores clínico-patológicos tradicionales con el método de 70 genes de MammaPrint. En este ensayo, si ambos métodos clasifican el riesgo de recaída del paciente como bajo, no se aplicó la quimioterapia adyuvante; si ambos métodos clasifican el riesgo de recaída del paciente como alto, se aplicó la quimioterapia; si los métodos daban resultados discordantes, el paciente fue aleatorizado para seguir el método clínico-patológico o seguir los resultados genómicos. En una primera instancia se determinó que un total de 1.550 pacientes (23,2%) tenían un riesgo clínico alto y un riesgo genómico bajo. En este grupo, luego de 5 años[5], la tasa de supervivencia sin metástasis a distancia entre los que no recibieron quimioterapia fue del 94,7%. La diferencia absoluta en esta tasa de supervivencia entre estos pacientes sin quimioterapia y los que sí recibieron quimioterapia fue de 1,5%, siendo la tasa más baja sin quimioterapia. Tasas similares de supervivencia sin metástasis a distancia fueron observadas en el subgrupo de pacientes que tenían cáncer de mama con RH + / HER2 - y enfermedad con ganglios negativos o ganglios positivos[5].

A continuación, el Dr. Petracci presentó los resultados de un seguimiento actualizado del estudio MINDACT que alcanzó una media de 8,7 años[6]. Incluyó un análisis exploratorio de un posible efecto de la edad (≤ 50 años frente a > 50 años) y un análisis por estado ganglionar para pacientes con enfermedad con RH + / HER2 -. Estos análisis se realizaron en la población por intención de tratar. En este seguimiento más maduro, la firma genética de MammaPrint mostró una robusta capacidad para identificar un subgrupo entre las mujeres con alto riesgo clínico, a saber, pacientes con un bajo riesgo genómico, con una excelente supervivencia libre de metástasis a distancia cuando reciben tratamiento con terapia endocrina sola.Para estos pacientes, la magnitud del beneficio de agregar quimioterapia a la terapia endocrina sigue siendo pequeña (2,6%) y no aumenta con la presencia de ganglios positivos.Sin embargo, en un análisis exploratorio de poca potencia, este beneficio parece depender de la edad, ya que en mujeres menores de 50 años se observa un beneficio de la quimioterapia de 5%. Este fenómeno podría estar relacionado en parte con la supresión de la función ovárica inducida por la quimioterapia[6].

También el Dr. Petracci mostró datos del ensayo IKA donde MammaPrint permitió identificar un subgrupo de pacientes postmenopáusicas con cáncer de mama no metastásico, con ganglios negativos y que tendrían una larga supervivencia sin recurrencia. La firma de 70 genes en estos pacientes con este tipo de cáncer de mama tan indolente presentaría un puntaje ultra-bajo y no requerirían el curso completo de terapia endócrina[7]. A continuación, presentó datos del ensayo NBRST, un estudio de prospectivo de fase IV en el que se evalúan los resultados del régimen de quimioterapia neoadyuvante y terapia endocrina neoadyuvante, tanto como respuesta al tratamiento en el momento de la cirugía como a más largo plazo a los 5 años[8]. Dado que los tumores se clasifican por matriz de expresión génica con el perfil de subtipificación molecular BluePrint y el perfil de pronóstico MammaPrint, se pudo comparar la respuesta al tratamiento de acuerdo con la clasificación clínica convencional con la molecular. Con

la subtipificación molecular por BluePrint, el 18% de los pacientes clínicos "luminales" se clasifican en un subgrupo diferente, en comparación con la evaluación convencional, y estos pacientes tienen una tasa de respuesta significativamente mayor a terapia endocrina neoadyuvante en comparación con los pacientes BluePrint "luminal". La subtipificación molecular MammaPrint/BluePrint puede ayudar a asignar un tratamiento eficaz a los pacientes adecuados. Además, la identificación precisa de la biología del subtipo es importante en la interpretación de la respuesta al tratamiento neoadyuvante, ya que la falta de respuesta completa patológica en pacientes luminales no presagia el peor pronóstico asociado con la enfermedad residual en los subtipos basal y HER2[8].

Podemos concluir diciendo que los trabajos aquí presentados contribuyen a abordar uno de los principales desafíos en oncología, que es la capacidad de distinguir con precisión entre los pacientes que necesitan tratamiento adyuvante y los que no. Esto, junto con la identificación del mejor tipo de terapia para el paciente individual, son algunos de los objetivos de la medicina personalizada, que es el rumbo hacia el que apuntan estas nuevas tecnologías

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Mesa redonda 2 (Sesión 8): Biorrepositorios y gestión de muestras

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Resumen

En esta mesa redonda se abordaron una variedad de temas relacionados con los biobancos. Se discutió, desde una perspectiva biopolítica de los biobancos para investigación, la tensión que genera en el marco de la globalización, el concepto de bien común y la forma de participar en el espacio público, planteando el concepto de soberanía genómica. Se abordaron aspectos técnicos y de gestión de muestras para la implementación de un biobanco, la necesidad de definir qué bioespecímenes se almacenarán, la infraestructura, recursos humanos, procedimientos operativos estandarizados, requisitos bioéticos, programas de bioseguridad, de contingencia, de gestión de calidad y de auditoría interna. Luego escuchamos la experiencia del biobanco en el *Moores Cancer Center* de la UCSD, marcando un contraste con nuestra realidad, pero también muy enriquecedor, ver la participación del biobanco en proyectos de investigación clínica, como forma de generar recursos para la sustentabilidad. Posteriormente, fue presentada la Red de Biobancos de Latinoamérica y el Caribe (REBLAC), se recorrió su historia, su crecimiento, los países participantes y sus actividades de promoción, capacitación y apoyo al desarrollo de los biobancos con fines de investigación. Por último, se presentó la "Guía para biobancos de muestras biológicas de origen humano con fines de investigación", recientemente aprobada por el Ministerio de Salud con la Resolución 2940/2020, que trata de establecer criterios unificados para el funcionamiento de los biobancos, armonizando criterios y brindando seguridad a los actores. aumentando la confianza en los donantes y la comunidad, que, con sus muestras, son el motor de los biobancos.

Abstract

Round Table 2 (Session 8): Biorepositories and sample management. This round table addressed an interesting variety of topics related to biobanking. From a biopolitical perspective of research biobanks, the group discussed the tension generated in the context of globalization, the concept of common good, the way to participate in the public space and the concept of genomic sovereignty. The technical and sample management aspects, the need to define which biospecimens will be stored, infrastructure, human resources, standardized operating procedures, bioethical requirements, the need to have biosafety, contingency, quality management and internal audit programs were also discussed. The experience of the biorepository at the Moore Cancer Center of the University of California in San Diego, was in stark contrast with our reality, but also very enriching to see the participation of the biorepository in clinical research projects, as a way of generating resources for the sustainability of biobanks. The Latin American and Caribbean Biobanks Network (REBLAC) presented its history, its growth, the participating countries and its activities of promotion, training and support to the development of biobanks for research purposes that REBLAC has in the region. Finally, the "Guide for biobanks of biological samples of human origin for research purposes", recently approved by the Ministry of Health with Resolution 2940/2020, the product of several years of work by a group of experts from the Ministry of Science and Technology, was presented. Harmonizing criteria and

providing security to the actors, increasing confidence in donors and the community, who, with their samples, are the driving force to establish unified criteria of biobanks.

Esta mesa redonda fue presidida por el Dr. Eduardo Sandes del Instituto Roffo, Buenos Aires; y copresidida por la Dra.Fabiana Lubieniecki del Hospital Juan P. Garrahan, Buenos Aires, Argentina. El objetivo de esta sesión fue mostrar el estado del arte de la implementación de Biobancos (BBs) en Argentina para su uso en investigación en cáncer, entre ellos cáncer de mama.

La primera disertante fue la **Dra. Liliana Virginia Siede** de la Universidad de Buenos Aires (UBA) y Universidad del Museo Social Argentino (UMSA), Buenos Aires, Argentina y nos habló sobre las **"Perspectivas biopolíticas y bioéticas de los Biobancos para investigación con muestras biológicas humanas"** quien encaró el tema de los BBs desde un punto de vista social. Mencionó que partimos de un mundo global que se sustenta en una expansión económica internacional mediada por el concepto de desarrollo biotecnocientífico y la tecnología de la información (sociedad informacional). Como consecuencia hay una fragmentación del mundo en función de recursos, estructuras y acceso de los diferentes países y que en su proceso de ciencia no siempre representa mayor bien común para todas las poblaciones. De allí que con la globalización se pone en tensión el concepto de bien común y la forma en que se participa en el espacio público.

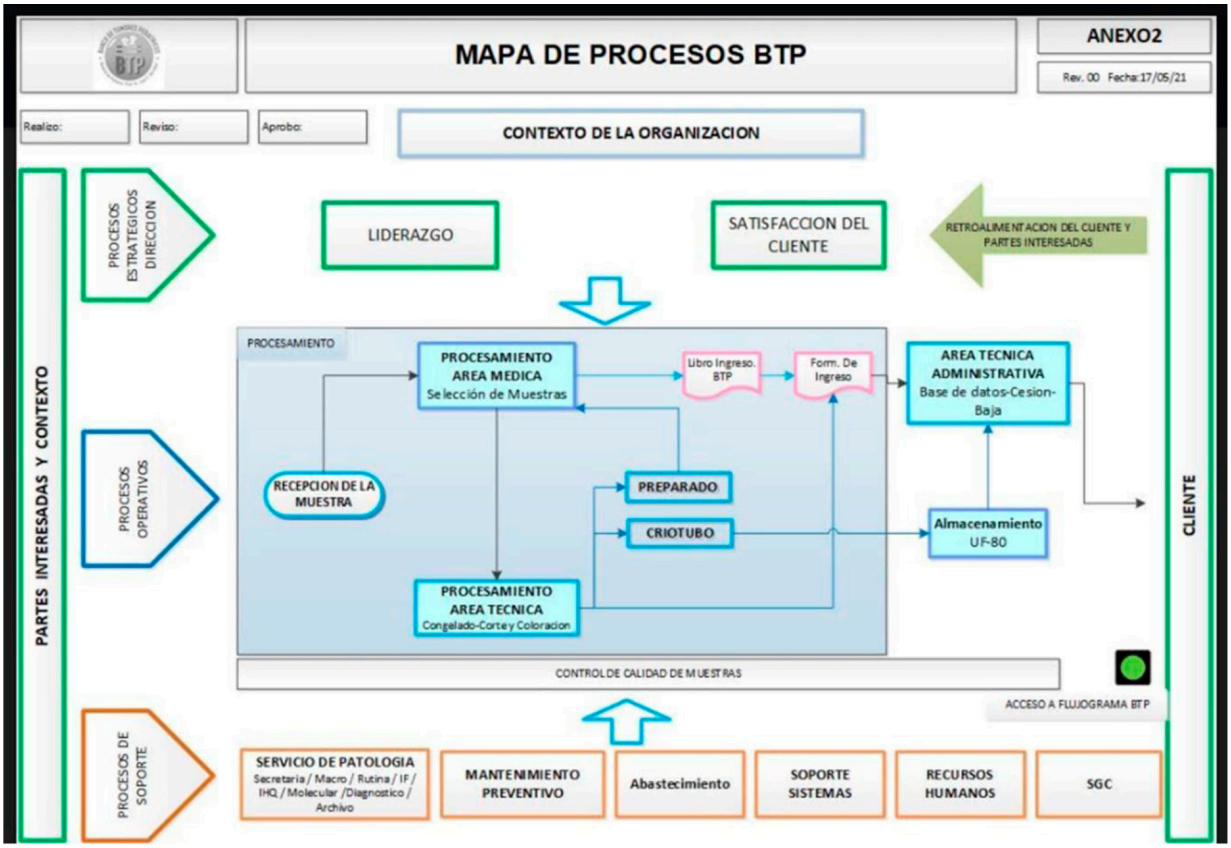
En este marco los BBs para investigación con muestras biológicas humanas almacenan el capital biológico de la población con estatus de recursos nacionales. Constituyen una parte esencial de la infraestructura de la biotecnociencia global, donde los límites territoriales de los estados se diluyen. Por otro lado, la investigación con muestras biológicas humanas requiere del consentimiento informado (CI) para proteger a cada persona, eje fundamental para la ciencia. Desde el principio de respeto a la autonomía, los derechos individuales están protegidos como valor ético fundamental, pero no están protegidos los derechos colectivos de la población incluidas las generaciones futuras. Cabe señalar que el CI representa la voluntad de los sujetos participantes mientras que el alcance de la información genómica que se investiga representa el interés de las poblaciones. Estos recursos nos obligan a repensar la forma en que se protegen los bienes comunes. Aquí la pretensión de esta presentación trae a la Biopolítica para pensar y reflexionar, que como dice Michel Foucault[1] por primera vez en la historia, lo biológico se refleja en lo político tanto como los procedimientos de saber y poder que se expresan a través del cuerpo de las personas, en la salud individual y colectiva, en las probabilidades de vida y en las condiciones de existencia. Otros autores, como Negri y Hardt[2] nos llevan a repensar el bien común que representa la ciencia proveniente de los Biobancos a partir del concepto de gobernanza tomando en cuenta el cambio de paradigma que va de lo local a lo global proponiendo una nueva estrategia de poder que incluya al colectivo de la población. Así, Nikolás Rose, exponente de la biopolítica molecular, habla de una ciudadanía biológica a nivel local, nacional y trasnacional, que surge de los BBs, pensando en su protagonismo en defensa de los derechos de su salud, tomándolos en cuenta, más allá de ser proveedores de muestras biológicas[3]. Finalmente, desde la Bioética autores como Apel, Kotow y Maliandi piensan en la sociedad como actor fundamental, protagonista en ejercicio de sus derechos a la salud, proponiendo una Bioética política que vaya a los consensos con la sociedad, más allá de las declaraciones éticas internacionales y principios[4].

La siguiente presentación **"Procedimientos operativos en banco de tumores y gestión de muestras"** estuvo a cargo de la **Dra. Andrea Bosaleh** del Hospital Nacional de Pediatría Juan P. Garrahan, Buenos Aires, Argentina**.** La presentación se basó en su experiencia en el Banco de Tumores Sólidos Pediátrico del Hospital Garrahan, primer BB de tumores público del país, con 15 años de trayectoria. Definió un BB como una unidad funcional sin fines de lucro en una institución pública o privada, que almacena una o varias colecciones de bioespecímenes de origen humano, datos personales e información asociada y que está organizada conforme con normas técnicas con criterios de calidad, orden y destino[5]. El objetivo de los BBs es distribuir a investigadores, muestras con información asociada, colectada, procesada y almacenada con criterios de calidad estandarizados[5]. La obtención de material en fresco provocó un cambio de paradigma, se pasó de tener tacos parafinados a muestras congeladas, debiendo definirse el soporte utilizado: un criomolde o un criotubo. Antes de su implementación, los BBs deberán establecer: si colectarán solo muestras de pacientes o también de voluntarios sanos; qué bioespecímenes se almacenarán (sangre y hemoderivados, otros fluidos corporales, ácidos nucleicos, tejido tumoral) y qué parámetros clínicos serán los datos asociados. Se establecerán las normas éticas y legales, como el CI, el comité de ética en investigación (CEI) asociado al BB, los recursos humanos, la infraestructura y el equipamiento e insumos para su funcionamiento. Las áreas de colección, recepción, manipulación y almacenamiento deberían estar, preferentemente, separadas y con acceso restringido. El personal técnico y administrativo debe ser idóneo para garantizar la integridad física de las muestras. Se establecerá la plataforma criogénica (Ultrafreezer, nitrógeno líquido), el volumen de las muestras y alícuotas, si se realizarán estudios posteriores (moleculares, genéticos, etc.), el *software* y *hardware* a utilizar en el manejo de la información, con un sistema de resguardo de ésta (anonimización, codificación), respetando la Ley de protección de datos personales (Ley 25.326/00). Los equipos de criopreservación requieren monitoreo continuo (temperatura y suministro eléctrico), con envío de alarmas al personal responsable. Se establecerán planes de bioseguridad, para manipulación y descarte seguro de residuos biológicos y material contaminado; de mantenimiento preventivo de instalaciones y equipamiento (matriz de equipos críticos); un plan de contingencia ante catástrofes y un plan de auditoría interna periódico que garantice el funcionamiento del BB[5].

Para trabajar en sistemas de gestión de calidad[6] se debe tener un mapa que ordene el trabajo o mapas de procesos (Fig. 1). Se dividen en tres ramas: estratégica (verde), operativa (azul) y procesos de soporte (naranja), estos mapas serán distintos para cada BB y cada uno deberá generar el propio.

La recepción de la muestra es un proceso administrativo, se le asigna un número y se registran 3 horarios claves: clampeo en el quirófano (1), ingreso al Banco de Tumores Pediátricos (2) y al ser almacenada (3), el tiempo entre 1-3 debe ser menor a 30 minutos. Se dispone de un formulario para datos demográficos y de registro de la cantidad de criotubos o criomoldes almacenados, al que se adjunta el CI. Para desarrollar un sistema de gestión de calidad, cada proceso debe estar documentado, en un mismo formato. En su BB se usan los dos tipos de soporte, los criomoldes son útiles porque preservan la

Fig. 1.– Mapa de procesos

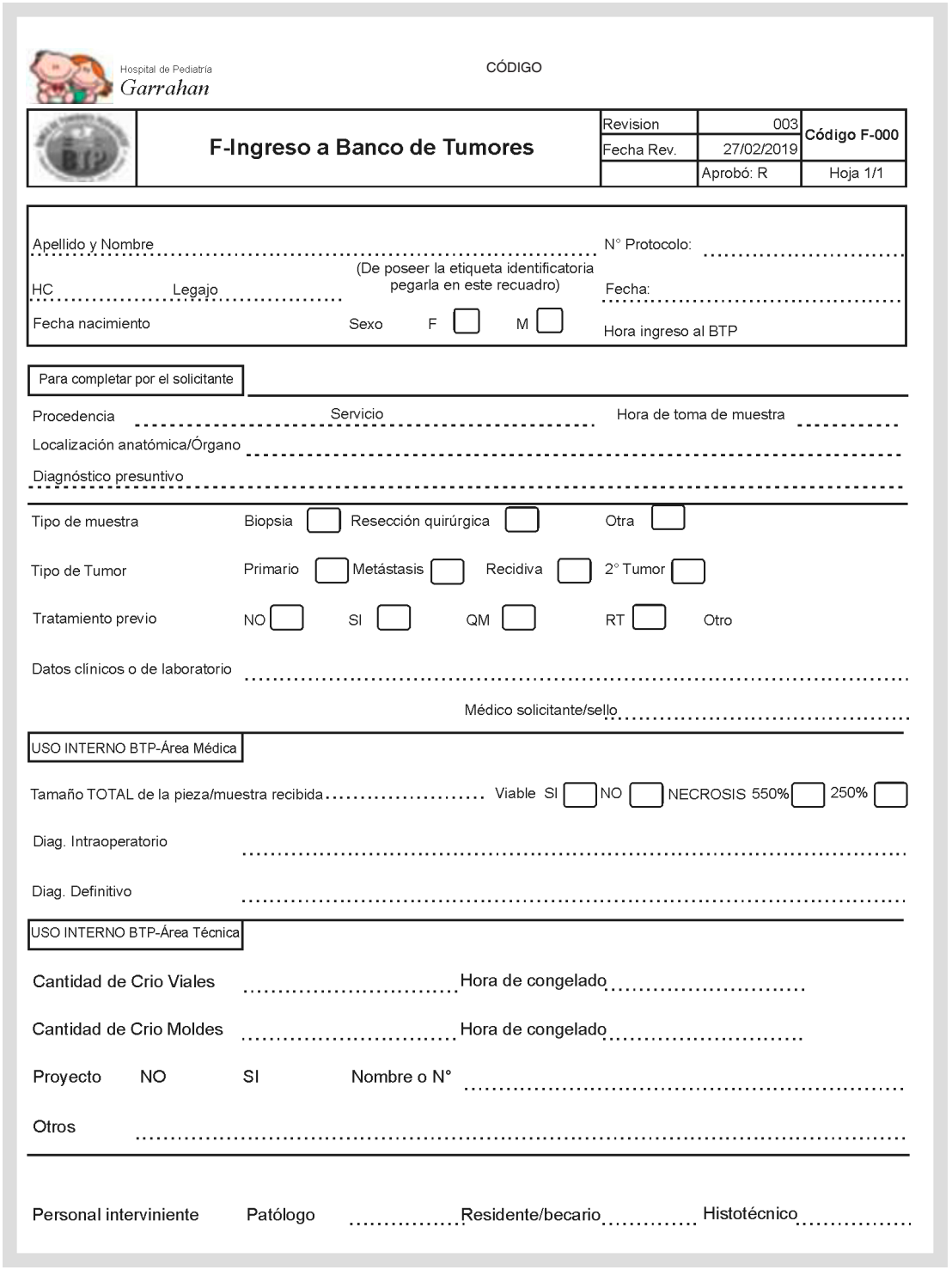


morfología y los criotubos permiten una mejor extracción de ácidos nucleicos. Se evalúa en la muestra, la viabilidad, la presencia o no de tumor y de necrosis (<50%, >50% y <80% y >80%), esto se registra en un formulario (Fig. 2) y según ciertos criterios se hace una guarda temporal. La congelación rápida, es por inmersión en isopentano a -80°C/-150°C, o en N_2 líquido/gas, ésta presenta el inconveniente de formar cristales, luego se almacena en ultracongelador. Es necesario tener documentado y estandarizado, el registro del almacenamiento de cada muestra, para su correcta trazabilidad, para gestionar esta información, se pueden utilizar desde planillas de cálculo (Excel), hasta sofisticados programas comerciales. El control de calidad se realiza anualmente, en el 1% de las muestras de forma aleatoria.

Finalmente, existen guías para desarrollar un sistema de gestión de calidad en un BB[6,7], esto es fundamental, por la necesidad de disponer de muestras biológicas de alta calidad. La colección, procesamiento y almacenamiento en forma estandarizada permitirá la reproducibilidad de resultados en futuras investigaciones, favoreciendo a futuro, el trabajo en red.

El tercer disertante fue el Dr. Alfredo Molinolo, Director del Biorepositorio del Moores Cancer Center, Universidad de California San Diego (UCSD), CA, Estados Unidos, y habló sobre "**La experiencia del *UC San Diego Biorepository and Tissue Technology Shared Resource (BTTSR)*".** El BTTSR del *Moores Cancer Center* (MCC) de la UCSD, es un BB con un laboratorio de patología asociado. Es una unidad independiente de patología, tiene un proyecto propio aprobado por el *Institutional Review Board* o CEI. El BTTSR cuenta, además del Dr. Molinolo como director general, con 3 directores asistentes, uno en la parte clínica, otro en el mismo biorrepositorio y otro del área en histología e histopatología. Tienen un asistente de patólogo, técnico en patología, quien recolecta las muestras y las lleva para ser almacenadas. No se procesan las muestras dentro del BB. Hay cuatro coordinadores que entrevistan a los pacientes y se ocupan de realizar los CI y cuatro histotecnólogos que procesan las muestras. Todo el personal es a tiempo completo, Cuentan también con estudiantes que colaboran en diferentes tareas. Tienen proyectos de investigación propios, publicando como biorrepositorio, independientemente de lo que publiquen como investigadores. Existen programas y estudios relacionados, exclusivamente, con técnicas de biorrepositorio. En infraestructura, poseen laboratorios y oficinas con más de 15 ultracongeladores y 2 tanques de nitrógeno líquido con capacidad para 40.000 muestras cada uno. Histopatología tiene su propio espacio. Se trata de un laboratorio que hace el 90% de la histopatología experimental de la universidad y el 20% de la investigación clínica. Está totalmente automatizado y hace diseños de *tissue microarray*, escaneo de vidrios, *multiplexing*, inmunofluorescencia, inmunohistoquímica, etc. También han generado una colección de bioespecímenes experimentales, entre ellos *patient derived xenografts* (PDXs). Como biorrepositorio tienen una colección de tejidos congelados y una colección

Fig. 2.– Formulario de ingreso de la muestra



Hospital de Pediatría *Garrahan*

CÓDIGO

	F-Ingreso a Banco de Tumores	Revision	003	Código F-000
		Fecha Rev.	27/02/2019	
			Aprobó: R	Hoja 1/1

Apellido y Nombre ..... N° Protocolo: .....

HC ..... Legajo ..... (De poseer la etiqueta identificatoria pegarla en este recuadro) Fecha: .....

Fecha nacimiento Sexo F ☐ M ☐ Hora ingreso al BTP

Para completar por el solicitante

Procedencia ..... Servicio ..... Hora de toma de muestra .....

Localización anatómica/Órgano .....

Diagnóstico presuntivo .....

Tipo de muestra Biopsia ☐ Resección quirúrgica ☐ Otra ☐

Tipo de Tumor Primario ☐ Metástasis ☐ Recidiva ☐ 2° Tumor ☐

Tratamiento previo NO ☐ SI ☐ QM ☐ RT ☐ Otro

Datos clínicos o de laboratorio .....

Médico solicitante/sello .....

USO INTERNO BTP-Área Médica

Tamaño TOTAL de la pieza/muestra recibida ..... Viable SI ☐ NO ☐ NECROSIS 550% ☐ 250% ☐

Diag. Intraoperatorio .....

Diag. Definitivo .....

USO INTERNO BTP-Área Técnica

Cantidad de Crio Viales ..... Hora de congelado .....

Cantidad de Crio Moldes ..... Hora de congelado .....

Proyecto NO SI Nombre o N° .....

Otros .....

Personal interviniente Patólogo ..... Residente/becario ..... Histotécnico .....

importante de tejidos en parafina, si bien, la mayor parte de estos últimos están en Patología.

El BTTSR, maneja casi todos los ensayos clínicos y estudios de investigación de la Universidad y son un punto de contacto entre investigadores, brindando información sobre qué otros proyectos se realizan en la misma. Tienen mucho entrenamiento en descubrimiento de biomarcadores, brindan cursos universitarios de patología e histopatología y uno específico de biorrepositorios, obligatorio para los coordinadores de ensayos clínicos. Recientemente han desarrollado un protocolo de congelamiento de células viables, y publicaron el desarrollo de líneas celulares y PDX a partir de 5 especímenes almacenados en nitrógeno líquido[8]. Un porcentaje elevado del presupuesto anual, alrededor del 60%, proviene del *Cancer Center Support Grants (CCSGs) for NCI-designated Cancer Centers (P30)* mientras que el resto proviene de servicios que los investigadores pagan a partir de sus subsidios. El BB no guarda extensa información de pacientes, porque tiene pleno acceso a las bases de datos de la Universidad, la seguridad de la información está en manos de ella. El centro del trabajo es el *Research Branch* con su programa de protección de humanos, revisado por el CEI, donde deciden todo lo que van a ceder. Algo muy importante es el CI, es un CI amplio y lo hacen en ocho idiomas, (inglés, castellano, chino, ruso, vietnamita, hindi, tagalo y farsi), en función de las etnias que viven en San Diego y que se atienden en el MCC.

Para finalizar, el Dr. Molinolo se refirió, a que esencialmente realizan estudios prospectivos planificando la obtención de especímenes según lo que el investigador quiere que se colecte y, además, realizan una adquisición aleatoria que depende de los intereses personales de los directores del BB.

A continuación, el **Dr.Gonzalo Ardao,** del Hospital Central de las Fuerzas Armadas (HCFFAA), Montevideo, Uruguay disertó sobre la "**Red de Biobancos de Latinoamérica y el Caribe (REBLAC)".** Mencionó que años atrás, un grupo de profesionales interesados en la preservación de tejidos congelados con fines de investigación, crearon una red cuyo objetivo era la armonización de diferentes procesos en los BBs, con una fuerte impronta en los requerimientos éticos, los procedimientos técnicos y buenas políticas de gestión de calidad, para apoyar proyectos de investigación colaborativos entre los países. La REBLAC está organizada en una junta directiva con un Coordinador, un vicecoordinador, tesorero, secretario y vocales, que se apoya en una comisión científica con una asesoría jurídica, una asesoría en bioética y una asesoría científica. Existen diferentes grupos de trabajo para: capacitación y entrenamiento, con 4 centros de referencia (Méjico, Colombia, Brasil y Argentina), visitas de evaluación técnica a BBs nuevos, creación de talleres y reuniones generales/asambleas, armonización de los procesos, evaluación de los estatutos, los requerimientos mínimos para cada BB, la información clínica y epidemiológica asociada y la gestión de calidad. La REBLAC se inicia en el 2007, logrando la primera reunión en Brasil-2008, conformándose con representantes de cinco países, Colombia, Ecuador, Brasil, Uruguay y Venezuela. Luego, se incorporaron BBs de otros países, con las reuniones en Colombia-2009, México-2010, Argentina-2011 y Uruguay-2012, Ecuador-2014, Perú-2016 y Colombia-2019. Además, la Red participó en eventos internacionales como el séptimo Congreso Nacional de Biobancos en España-2016, realizando el Primer Congreso Latinoamericano de Biobancos. Actualmente, Argentina tiene cuatro BBs y Brasil tiene tres y se efectuaron cursos y visitas de intercambio en los diferentes países, con actividades de entrenamiento y capacitación. La mayoría de las instituciones participantes son públicas, unas pocas son público/privada o privadas y un gran número colectan muestras histopatológicas y/o de sangre de pacientes oncológicos adultos y/o pediátricos. Algunos BBs colectan muestras de individuos sanos, junto a datos demográficos, y guardan información clínica asociada, y otros utilizan la base de datos del propio hospital. Ciertos BBs colectan, además, muestras de tejido "normal", entendiéndose a éste como tejido de la pieza de resección del paciente, alejada del tumor y macroscópicamente normal. Para almacenamiento, la mayoría utiliza criotubos y para criopreservar algunos usan nitrógeno líquido y otros, para reducir costos, utilizan ultracongeladores mecánicos. Los tumores colectados más frecuentemente en la red son: cáncer de mama, tiroides, colorrectal y en menor grado cáncer gástrico y de endometrio. Operativamente, todos cuentan con sistema de soporte eléctrico de emergencia y la mayoría con sistema de monitoreo de temperatura. Todos poseen procedimientos operacionales estándar y algunos tienen certificación de calidad ISO-9000 o acreditación por entidades locales, junto a programas de gestión de calidad y sistemas de gerenciamiento de muestras e información de los pacientes.

Finalizando mencionó que esta es la historia de la REBLAC cuyo objetivo es trabajar solidariamente con los biobancos de Latinoamérica y el Caribe para armonizar procedimientos y ayudar a mejorar a aquellos que están en formación, con el objetivo de poder trabajar en red, entre los países de la región, generando muestras de calidad.

Por último, la Dra. **Ana Palmero** del Ministerio de Salud de la Nación, Buenos Aires, Argentina se refirió a los **"Biobancos con fines de investigación: Experiencia de la elaboración de pautas éticas en Argentina":** La Comisión Ad Hoc de BBs del Ministerio de Ciencia y Tecnología (MinCyT), luego de varios años de trabajo desarrolló las Recomendaciones para BBs con fines de investigación[5]. Sobre esta base, el Ministerio de Salud (MSAL), por intermedio del Área de Ética en Investigación, a cargo de la Dra. Palmero, aprobó la "Guía Para Biobancos De Muestras Biológicas De Origen Humano Con Fines De Investigación", con la Resolución 2940/2020, abordando aspectos éticos, técnicos y legales para el funcionamiento de BBs[8]. En Argentina, existe la Guía para Investigación en Salud Humana (MSAL-Resolución 1480/11) y la ANMAT regula los ensayos clínicos; sin embargo, no existía una normativa para BBs con fines de investigación, dificultando su operatividad, por falta de claridad en aspectos éticos y legales de las muestras almacenadas. Algunas controversias hacen referencia al uso de un CI amplio o específico, la utilización de muestras de personas fallecidas o que no tienen CI previo, la discusión sobre la propiedad de las muestras, qué actividades se pueden realizar y qué autorizaciones se precisan para realizarlas. El MSAL intenta regular y alcanzar un equilibrio entre promocionar la existencia de los BBs reportando un beneficio social y proteger los derechos de los donantes y de la comunidad, que ceden sus muestras. Orienta a investigadores, operadores de BBs, patrocinadores, miembros del CEI, autoridades reguladoras y sanitarias, sobre las pautas éticas aplicables a los BBs con muestras humanas y sus datos asociados, facilitando su implementación y funcionamiento. La guía da una definición internacional de BB y establece que el ámbito de aplicación son los BBs con fines de investigación, que el BB es el custodio y guardián de las muestras, no es

el dueño, sino el responsable de garantizar la calidad de lo almacenado, asegurando el respeto de los derechos e intereses de los donantes y sus comunidades. Establece que los BB son sin fines de lucro, las muestras no tienen un valor comercial, pero sí, pueden estipularse cargos de recupero por el mantenimiento, la logística y todo lo que implica el funcionamiento del BB.

La guía está organizada según los distintos momentos de la muestra: recolección, almacenamiento, cesión y utilización posterior en investigaciones. Respecto a la recolección, establece pautas para el proceso del CI, promoviendo el CI amplio, ya que se desconoce el uso futuro de la muestra, evitando la complicación del CI especifico, de contactar al donante para acciones futuras y establece límites en los usos según los intereses y preferencias de los donantes, garantizando evaluaciones periódicas del CEI. Sobre el almacenamiento, el BB debe garantizar la confidencialidad de los datos de los donantes (Ley 25.326/00), con la anonimización de las muestras y sus datos asociados, siendo éste quien posee el vínculo entre la muestra y los datos. Propone una comunicación abierta entre BB, donantes y comunidad, informando las investigaciones que se hacen, cuándo contactar nuevamente al donante, cómo informar a la comunidad y cómo planificar las comunicaciones. Establece pautas para los acuerdos de transferencias de materiales (ATM). Los trabajos colaborativos internacionales, poseen ATMs preconfeccionados y las instituciones pueden carecer de las capacidades para evaluarlos, definir sus derechos y los beneficios posteriores, estableciendo elementos mínimos para cerrar estos acuerdos, definiendo las obligaciones entre el proveedor y el receptor respecto al uso de las muestras, la protección de los intereses de donantes y comunidad, garantizando asociaciones justas y colaborativas entre receptor y proveedor. El acceso a los beneficios debería definirse en el ATM, tanto para las comunidades, como para el BB, que haya un reconocimiento al BB que aportó las muestras y una devolución de los datos y los resultados de estas investigaciones para comunicar a la comunidad, los ATM podrán incluir entrenamiento del personal o capacidades que precise el BB. También propone tener un CEI asociado al BB, que tenga una relación y lo conozca, generando evaluaciones más expeditivas. Es importante que exista un proceso de revocación del CI real, que, si el donante desea retirar sus muestras y retirar sus datos, estos procedimientos sean de fácil acceso.

Por último, esto es un punto de partida que intenta establecer criterios para el funcionamiento de los BB, armonizando criterios y brindando seguridad a los actores, aumentando la confianza en los donantes y la comunidad, que, en definitiva, son los proveedores de muestras que hacen funcionar los BB y las investigaciones[10].

Luego de las 5 presentaciones se originó un debate en el cual se discutieron las inquietudes de los investigadores, en general del campo de investigación en cáncer de mama donde se puntualizó que todavía no existen bancos en los cuales se puedan solicitar muestras ya que los mismos son usados por los hospitales donde funcionan o no son de libre acceso para a la comunidad científica. Por otro lado, se mencionaron las limitaciones que ponen los CEI a la hora de presentar proyectos basados en el uso de muestras de Biobancos por lo cual falta todavía un camino a recorrer. Quedó claro que el ideal podría ser alcanzar los estándares como los del *Moore Cancer Center* en San Diego. Pero en términos puntuales como una primera etapa debemos lograr quizás constituir una Ley de Biobancos a nivel nacional que permita por un lado que los consentimientos informados que firman los pacientes contemplen la preservación de material en un BB para investigación. Por otro lado, que los BB que se establezcan en instituciones públicas con fondos públicos sin fines de lucro, puedan ser de acceso abierto con el cobro por muestra que sea destinado al mantenimiento del servicio y cuyo requisito sea solamente contar con la aprobación por parte de Comités de Ética independientes en el marco de la Ley de BB establecida.

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Mesa redonda 3 (Sesión 11): Interacción entre agencias gubernamentales y no gubernamentales con la industria para financiar y promover la investigación traslacional en cáncer

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Resumen

El jueves 20 de mayo de 2021, dentro de las actividades del Buenos Aires*, Breast Cancer Symposium*, se desarrolló esta Mesa Redonda que contó con un distingui-

do panel de cuatro especialistas, de formación y actividad con alta pertinencia a la temática propuesta, cada uno con una visión singular del tema. La Dra. Judith Naidorf discutió la visión desde las Ciencias Sociales de los Proyectos de Desarrollo Tecnológico y Social destacando su implicancia a futuro. Rosana Felice aportó su visión desde la industria y como esta puede imbricarse con la investigación de mesada. Por su parte, los Dres. Andrea Llera y Daniel Gómez apuntaron a describir su visión desde la experiencia tanto en la solicitud de fondos para investigación para sus proyectos particulares como en organismos de gestión de fondos. La Dra. Llera mencionó aspectos no tradicionalmente considerados al preparar las solicitudes o evaluar los proyectos y el Dr. Gómez comentó las perspectivas actuales de colaboración con la Unión Europea. Las presentaciones fueron seguidas de debate por intercambio de comentarios entre panelistas y los participantes conectados por la plataforma virtual ZOOM. El evento fue moderado por la Dra. Marina Simian, la Dra. Edith Kordon y el Dr. Omar Coso

Abstract

Round Table 3 (Session 11): Interaction among government, non-government agencies, and industry for funding and promoting breast cancer translational research. As part of the activities of the Buenos Aires Breast Cancer Symposium, a special session was assembled following the format of a "Round Table". A distinguished panel of four specialists, with training and activity highly relevant to the proposed theme, delivered presentations and then engaged in a debate showing their respective singular vision of the subject. Dr. Judith Naidorf discussed from the point of view of the Social Sciences, the Technological and Social Development Projects, highlighting their implications for the future. Rosana Felice contributed her vision from the industry and how it can positively interact with academia and research at the bench level. In addition, Drs. Andrea Llera and Daniel Gómez aimed to describe their acquired experience both in requesting research funds for their particular projects and in fund management organizations. Dr. Llera mentioned aspects not traditionally considered when preparing applications or evaluating projects, and Dr. Gómez commented on the current perspectives of collaboration endeavours with the European Union. The presentations were followed by debate and exchange of comments between panelists and participants connected by the ZOOM virtual platform. The event was moderated by Dr. Marina Simian, Dr. Edith Kordon and Dr. Omar Coso"

La primera presentación estuvo a cargo de la Dra. Judith Naidorf, quien habló sobre la movilización del conocimiento como marco de la investigación traslacional. La Dra. Judith Naidorf es Investigadora Independiente de CONICET en el Instituto de Investigaciones en Ciencias de la Educación de la Facultad de Filosofía y Letras, Universidad de Buenos Aires y es especialista en estudios sobre educación superior, estudios sociales de la ciencia y política científica.

La Dra. Naidorf presentó tres etapas de la investigación traslacional:

Del laboratorio a la cabecera del paciente. Practicando una traducción efectiva de los nuevos conocimientos y técnicas generadas mediante los proyectos de ciencias básicas hacia nuevos enfoques para la prevención, diagnóstico y tratamiento de las enfermedades.

De la Investigación a la práctica. Llevando los resultados de los estudios clínicos a la clínica diaria y a la toma de decisiones en salud.

Investigación de Nivel Político y de impacto Sanitario. Enfatizando las consecuencias ambientales de estos cambios de procedimiento, y efectos sobre política y sostenibilidad de estrategias de gestión y salud pública.

Judith Naidorf también aprovechó la oportunidad para presentar los proyectos tipo PDTS (**Banco Nacional de Proyectos de Desarrollo Tecnológico y Social)**. Este Banco es coordinado por el Ministerio de Ciencia, Tecnología e Innovación de la Nación, y fue creado en abril de 2014 a partir de los consensos institucionales generados en torno a los documentos I y II de la Comisión Asesora sobre Evaluación del Personal Científico Tecnológico. Los PDTS constituyen una estrategia interinstitucional para orientar las actividades del sistema científico argentino a la resolución de problemas de la sociedad y el medio social y productivo. Tiene como objetivo, además, visibilizar la investigación y producción de conocimiento con perfil tecnológico y de innovación social.

Entre las principales **conclusiones** de este emprendimiento pueden citarse: 1) convergencia de múltiples instituciones con necesidades de cambios en procedimientos manteniendo sus diferencias y las particulares formas de desarrollo profesional de sus científicos y científicas. 2) Inaugura la centralidad de la figura del "demandante". 3) Innovación en modalidades alternativas de evaluación académica. 4) Objetivos ambiciosos tales como "crear un nuevo perfil de investigador". 5) La mayoría de los proyectos tiene como beneficiarios a organismos públicos y pueden ser afectados por los cambios de gobierno. 6) La convocatoria CIN con financiamiento de 2014 resultó en un gran impulso. 8) No se constituyó en una línea de financiamiento alternativo para la comunidad académica. 9) Se verificaron aprendizajes tanto del sector académico como del social como resultado de la interacción con agentes sociales.10) Las y los académicos involucrados en PDTS no notaron cambios sustantivos en su evaluación académica y la mayor parte de ellas y ellos mantuvieron sus prácticas tradicionales por lo que la participación en PDTS se constituyó en una tarea adicional o paralela no valorada de la misma manera por comisiones evaluadoras del desempeño académico (concursos, informes, promociones, etc.).

La disertante mencionó como desafíos a futuro: 1) Fomentar la coproducción de conocimiento. 2) Encontrar formas alternativas de evaluación académica efectivas. 3) Promover, motivar y valorar la actividad científica no aplicada sino abordada de modo tal que la producción de conocimiento actúe como estrategia de movilización del conocimiento. 4) Emitir señales claras a la comunidad académica comprometida con el desarrollo social.

La segunda oradora fue la Dra. Rosana Felice, médica especialista en Farmacología Clínica que actualmente se desempeña como Directora Médica y de Investigación y Desarrollo en GlaxoSmithKline (GSK) para Argentina y cono sur. El tema de su presentación fue sobre la Cooperación público-privada en investigación básica y la experiencia de GSK en el programa *Trust in Science*. La disertante llevó adelante su ponencia bajo el lema "Aso-

ciación para la Cooperación" y planteó como idea general compartir la experiencia de GSK como compañía farmacéutica en la cofinanciación de proyectos de investigación con un organismo estatal. Describió aspectos generales de la empresa con 97 años en Argentina y 730 empleados en la actualidad.

GSK desarrolla el programa *TRUST IN SCIENCE (TIS)* que se ha sostenido pese a los cambios de gestión y tiene como objetivos posicionar a GSK como "*partner* de investigación" confiable y serio en Latinoamérica y contribuir mediante fondos e interacciones científicas con las más importantes instituciones locales.

Rosana Felice citó a Michael Rosemblatt quien antaño elogiaba como la academia y la industria farmacéutica pueden trabajar juntos trayendo el ejemplo de Merck Sharp and Dohme, interactuando con la Clínica Mayo y diversas universidades. Hoy,academia e industria colaboran en el desarrollo de vacunas en el contexto de la pandemia COVID-19. En todos los ejemplos citados el público ha sido el principal beneficiado.

De acuerdo a la presentación de Felice, existen múltiples ejemplos de contribuciones complementarias entre la academia y la industria. Mientras que la academia provee una comprensión profunda de la biología, las empresas tienen el potencial de hacer inversiones y aportar infraestructura. Este modelo bien establecido para la construcción de infraestructura económica y social, se emplea en más de la mitad de los países del mundo.

En estos contratos a largo plazo con el fin de proveer un servicio o un bien público el sector privado asume un riesgo grande y retiene la posibilidad del gerenciamiento. El modelo TIS se emplea desde el acuerdo de cofinanciación firmado con el gobierno de Argentina desde 2011. Similares emprendimientos han tenido sus experiencias con mayor o menor éxito en Brasil y países de África.

Durante su presentación Felice afirmó que de generarse propiedad intelectual la misma pertenece al investigador o a la institución. En Argentina se han desarrollado 33 proyectos de investigación desde 2011 con una inversión de más de 10 millones de dólares. Los mismos son supervisados mediante diálogos cada 6 semanas. En la historia de TIS en Argentina han participado más de 220 científicos, 21 postdocs, se han contribuido 91 publicaciones y existen 5 patentes en trámite. Todo esto ha contribuido a generar reputación favorablemente creciente de la compañía como "*partner* de elección" en Latinoamérica.

La disertante mostró la página WEB del último llamado en PCE GSK 2020 la cual puede ser consultada dentro del sitio WEB de la AGENCIA NACIONAL DE PROMOCION CIENTIFICA y TECNOLOGICA y una nota en la revista *Nature* elogiando el programa.

Terminó su alocución destacando dos conceptos: 1) la existencia de un alto grado de compromiso social, más allá del contrato. Para que las asociaciones sean exitosas se requiere compromiso personal, y 2) la idea de que cada parte tiene que estar comprometida como su contraparte en alcanzar los objetivos del otro, tanto como los propios.

La tercera disertante de la mesa redonda fue la Dra. Llera y nos habló sobre las necesidades y costos ocultos de la investigación translacional contando una experiencia del mundo real. La Dra. Andrea Llera es bioquímica y farmacéutica, doctora de la Universidad de Buenos Aires e investigadora independiente de CONICET. Es miembro del Comité Directivo de la *Latin American Cancer Research Network* y co-investigadora principal de su estudio Perfil Molecular del Cáncer de Mama, el cual estudia una cohorte de 1300 pacientes con cáncer de mama de 5 países latinoamericanos.

La Dra. Llera ha elegido hablar sobre cómo se subsidia la investigación traslacional basándose en la experiencia personal en el planeamiento y ejecución del ***US-Latin American Cancer Research Network*** **(LACRN)** dando a entender que el carácter de este tipo de investigaciones no termina de ser entendida por los organismos de financiación.

El proyecto de referencia que además tiene elementos de medicina de precisión (un modo de acercarse a la enfermedad, tratamiento y prevención que toma en cuenta la variabilidad individual en genes, medio ambiente y estilo de vida de cada persona) se inició en 2011.

El proyecto parte de la intención de generar biobancos así como infraestructura genómica como recursos nuevos para poder continuar a futuro. Hoy está establecido el proyecto que ha llegado a conformar una cohorte de 1300 pacientes con microarreglos practicados en 1071para el seguimiento, con solo 51 pacientes perdidos. Se ha establecido una línea de base descriptiva para el concepto de medicina de precisión y se ha establecido el concepto de diversidad, lo cual hace que este proyecto sea único respecto a otras cohortes en varios aspectos.

Centros de investigación, y centros de salud de 5 países incluyendo 28 instituciones han debido desarrollar modos de trabajo coordinados que instalen procedimientos de operación estandarizados (SOPs), mecanismos de consentimiento informado y un lenguaje de trabajo común entre profesionales de la salud y de la ciencia con formación y rutinas de trabajo diversas.

El desafío de consolidar ese grupo multidisciplinario (que hoy se entiende mucho mejor) implica la generación de tareas y procedimientos indispensables para el correcto manejo y almacenaje del material biológico (incluyendo la colección de información asociada) en las situaciones óptimas que los objetivos científicos requieren. Todo eso contemplando no generar atrasos en la dinámica de funcionamiento hospitalario y minimizando las potenciales molestias para los pacientes.

Médicos clínicos, patólogos, personal involucrado en cirugía, técnicos colectores y procesadores de datos, reclutadores de pacientes, epidemiólogos, así como los investigadores están involucrados en una trama de personas que constituyen un equipo multidisciplinario de dimensión y complejidad sin precedentes y que son inherentes al carácter traslacional del proyecto.

A esta tarea, que de por si reviste complejidad, deben sumarse toda una serie de factores humanos y de infraestructura que generan acciones que deben ser contempladas como costos relacionados con la investigación y que no están contempladas en los instructivos de las fuentes tradicionales de financiación. Los mismos deben ser incorporados al abanico de costos relacionados con los objetivos del proyecto si lo que queremos es establecer líneas de investigación traslacionales que permitan la operativa de múltiples centros (con sus propiedades de infraestructura propia) y la necesaria intervención de diversos actores en una interacción armónica entre los componentes que garanticen el desarrollo de las prácticas planeadas.

Todo esto genera una necesidad de recursos económicos en rubros que no están pensados aun en los proyectos ni estaban pensados al pedir fondos para el proyecto de referencia. La Dra. Llera señala con énfasis que los organismos de financiación tienen que entender que las necesidades de financiación de los proyectos de mesada son diferentes a los de este tipo de proyectos.

Se introdujo la figura de los "bionavegadores", figura desconocida hasta entonces, para poder seguir al paciente y acompañarlo en su tránsito hospitalario de modo que no se perjudique su salud ni el protocolo del proyecto científico. Fue necesario adaptar los SOPs a las realidades de los hospitales, contemplar heterogeneidades en la disponibilidad de medicamentos en diferentes localidades, diferente instalación eléctrica en cada hospital, así como capacidades de procesamiento de datos, considerando hardware, software y capacitación del personal. Los costos adicionales también incluyen la carga que genera a los pacientes este tipo de estudios al tener que trasladarse a su propio costo entre centros para practicar diferentes observaciones o intervenciones requeridas en instituciones cuyas capacidades son complementarias, pero no están concentradas.

En principio, y en base a la necesidad de ajustar todos estos parámetros, se decidió hacer un estudio observacional dejando la intervención para una segunda parte a desarrollar a futuro.

Sin embargo, el estudio resulta en que el beneficio no solo se da mediante la publicación de *papers* sino que las pacientes resultan tener un seguimiento más personalizado, lo cual en si implica una devolución del estudio hacia las mismas. En ese sentido la parte observacional del estudio termina teniendo un carácter traslacional implícito.

Por último, el Dr. Daniel Gómez, Doctor en Medicina de la Universidad de Buenos Aires, que ha sido Rector de la Universidad Nacional de Quilmes en el período 2004-2008 donde reviste como Profesor Titular, disertó sobre las posibilidades de financiación en la Unión Europea ya que actualmente se desempeña como Punto Nacional de Contacto del MINCYT ante la Unión Europea (UE).

El Dr. Gómez invita a analizar la participación de investigadores argentinos en aplicaciones a proyectos de financiación de la UE en Investigación traslacional. Desde hace 30 años la UE financia proyectos de investigación fuera del viejo continente y desde hace 22 años el MINCyT cuenta con una oficina de enlace a tal fin.

¿Cómo nace esta iniciativa?: La UE tiene en su territorio el 7% de la población mundial pero aun así no alcanza tasas efectivas de innovación y emprendimiento que incrementen su PBI.

La cooperación internacional que la UE fomenta tiene como objetivo crear consorcios internacionales que involucren instituciones europeas y extranjeras como actores, de modo tal de proveer de manera recíproca capacidades complementarias que redunden en resultados de beneficio a todas las partes.

Daniel Gómez destaca el programa Horizonte Europa 2021/2027 lanzado con la expectativa de que al terminar el resultado de la investigación haya permitido incrementar el PBI en un 0,3%.

Cada año la UE lanza convocatorias en 6 grandes áreas incluyendo "salud". El funcionamiento de las presentaciones, evaluación y ejecución de estos emprendimientos guarda similitudes y diferencias con la operativa del CONICET.

Las convocatorias de la UE son cerradas temáticamente, con títulos muy específicos, por ejemplo: "Nuevos medicamentos en cáncer de mama triple negativo". Un determinado grupo de investigación, en las convocatorias de algunos años, podrá encontrar (o no) temáticas en las que esté interesado. Podrá participar en tanto y en cuanto su especialidad caiga por dentro de la esfera del universo de proyectos requeridos.

Mientras que en CONICET los fondos son solicitados por individuos, en los proyectos de la UE se convoca a consorcios conformados por 3 instituciones de 3 países diferentes dentro de la UE como mínimo, pero no como máximo. Establecido un consorcio, puede entrar al mismo una entidad de Argentina, por ejemplo, como cuarto país asociado.

Las instituciones contribuyentes a constituir el consorcio no están limitadas a universidades o Institutos de investigación. Está muy bien vista la participación de PYMES y ONGs, así como agencias de publicidad. Es de la más alta relevancia política para el Parlamento Europeo poder ir demostrando la significancia de los logros parciales. Ingredientes positivos adicionales que el Dr. Gómez recomienda incluyen la participación de países del bloque de Europa oriental (dado que se los quiere incorporar a la europea con relevancia científica) y la presencia de igualdad de género que se desprenda de la propuesta.

Ante un llamado y formado un consorcio que se ajuste a las reglas de la convocatoria en curso, la presentación se evalúa por tres evaluadores en forma separada que luego emiten un dictamen final.

Más allá de los contenidos experimentales de la propuesta y de las pertinencias interactivas entre los componentes consorciales, los parámetros que se buscan son: precisión, claridad, impacto, viabilidad y sustentabilidad. Se busca también tener una estimación de cómo sigue el proyecto cuando la financiación se termine. De obtenerse una patente, la misma pertenece al investigador dado que, a la UE le interesa la riqueza generada a partir del conocimiento obtenido.

¿Cómo hace un Investigador Argentino para formar un consorcio? El Dr. Gómez hace especial énfasis en la búsqueda de colaboradores actuales o pasados para estimular desde Argentina la conformación del núcleo basal del consorcio basado en la UE. Existe un portal de la UE que anuncia los consorcios en formación. Alternativamente el componente argentino puede anunciarse en el portal informando sus cualidades o características distintivas.

El Dr. Daniel Gómez enfatiza que su rol de Punto Nacional de Contacto de la UE se refiere a las propuestas en el área de salud. La colaboración de Diego Galeano quien se ocupa de analizar la admisibilidad, los aspectos legales, los financieros y los de procedimiento es enfáticamente destacada. Ambos forman un equipo cuyo rol es facilitar la presentación, espera recibir consultas y pide que no dejen de contactarlo.

Nota: Se agradece a los organizadores del BA-BCS, a los panelistas participantes de esta mesa redonda y, en especial, a la Dra. Marina Simian y a la Dra. Edith Kordon por todo el trabajo involucrado en la convocatoria de los especialistas, ensamble y coordinación del evento.